University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

Dissertations and Student Research in Entomology

Entomology, Department of

Summer 7-28-2021

CHARACTERIZATION OF WESTERN CORN ROOTWORM (COLEOPTERA: CHRYSOMELIDAE) SUSCEPTIBILITY TO FOLIAR INSECTICIDES IN NORTHEAST NEBRASKA

Timothy B. Dang
University of Nebraska - Lincoln

CHARACTERIZATION OF WESTERN CORN ROOTWORM (COLEOPTERA: CHRYSOMELIDAE) SUSCEPTIBILITY TO FOLIAR INSECTICIDES IN NORTHEAST NEBRASKA

by

Timothy B. Dang

A THESIS

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Master of Science

Major: Entomology

Under the Supervision of Professor Lance J. Meinke

Lincoln, Nebraska

July, 2021

CHARACTERIZATION OF WESTERN CORN ROOTWORM (COLEOPTERA: CHRYSOMELIDAE) SUSCEPTIBILITY TO FOLIAR INSECTICIDES IN NORTHEAST NEBRASKA

Timothy B. Dang, M.S

University of Nebraska, 2021

Advisor: Lance J. Meinke

The western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), is a major pest of maize in the United States. A variety of tactics are used to manage this pest such as crop rotation, insecticides, and transgenic maize expressing insecticidal proteins from *Bacillus thuringiensis* (Bt). WCR populations are highly adaptive and have evolved resistance to these management tactics. Management options are limited as few new tactics are available. Research is needed to evaluate the value of existing tactics used within an integrated framework to manage densities/injury and mitigate resistance.

This study evaluated the field performance of formulated foliar insecticides (bifenthrin, chlorpyrifos) targeting adult WCR populations in northeast Nebraska during 2019-2020 with the focus on short and longer-term effects on density. Cohorts of fields were treated with a single application of foliar insecticide or left untreated as controls during the peak adult activity period, then sampled for WCR density before and after treatment. WCR densities, sex ratio, and proportion gravid females were not significantly different in treated and untreated fields prior to insecticide application. Results indicated within-season efficacy of insecticides was excellent as mean adult density was significantly reduced post-application in treated fields compared to control fields. An

emergence cage study was conducted the following season to document the effect of foliar insecticides on adult survival. Total adult emergence was significantly reduced the following season in treated fields. Results suggest a single, properly timed insecticide application can reduce build-up of WCR density in continuous maize which would also reduce selection pressure on Bt maize where resistance occurs. A positive relationship between the sampling methods, whole plant counts and unbaited Pherocon AM sticky traps, was derived from the sample data.

Susceptibility of adult WCR populations in northeast Nebraska was further characterized by conducting laboratory vial bioassays with bifenthrin, chlorpyrifos, and dimethoate to develop dose-response curves. Results confirmed that the WCR populations were relatively susceptible to the active ingredients with exception of a few populations exhibiting low levels of resistance. These data will serve as a baseline for comparison in future bioassays and inform WCR management programs.

ACKKNOWLEDGMENTS

I would like to thank my academic advisor, Dr. Lance J. Meinke. His presence was an inspiration and his mentorship helped me grow as a scientist. I appreciate the opportunities he presented me during my graduate program to attend professional meetings and present my own talks. My time as part of his team exposed me to many settings and expanded my view on entomology and the world. Under his guidance, I greatly improved my writing, oratory, and critical thinking skills that are necessary for my future.

I would also like to thank the members of my committee, Dr. Ana María Vélez Arango and Dr. Robert Wright. Without their feedback, insight, ideas, and resources I would never have been able to finish my research. Dr. Ana Vélez was immensely helpful, providing me a viewpoint from a toxicological standpoint and teaching me about the field of toxicology. Dr. Robert Wright was a great wellspring of knowledge on the topic of corn rootworm and integrated pest management.

Special thanks to Miguel Fudolig, a Ph.D. student from the Department of Statistics and SC3L Helpdesk Consultant. He played a pivotal role in the data analysis portion of my research. He was extremely understanding and helpful, going out of his way to accommodate my busy schedule. Emily Robinson, a Ph.D. student from the Department of Statistics and SC3L Helpdesk Consultant, also aided in the analysis of a portion of data relevant to my research and deserves special mention.

Thanks to Dr. Arnubio Valencia Jimenez for willing to mentor me in vial assay procedures and helping develop the procedure for my research. I greatly appreciate his willingness to aid me in my research and his prompt response to my many questions. His advice and suggestions were essential in overcoming many challenges I faced while carrying out the laboratory experiments.

Both Jordan Reinders, a fellow graduate student under the mentorship of Dr. Lance J. Meinke and Ph.D. student, and Emily Reinders, the research technician for the lab deserves my heartfelt gratitude for their assistance both in the field and in the laboratory. I appreciate the help they gave me on the many trips to the many field sites over the summer, their help played an essential part of my success. Emily's assistance and experience in raising western corn rootworm populations was important in making my research successful.

I wish my fellow graduate students' success in your future endeavors and will always be cheering for you. Thank you for your friendship and support, for giving me great memories of my time here at UNL.

And finally, I would like to thank my family and relatives. Their love and support are what encouraged me to continue undaunted to the end of my program. Even from a great distance, my family never failed to keep in touch with me during holidays and special events; never failing to cheer me up. My uncle and his family were a great help for a comfortable transition from my hometown to an unfamiliar environment. He never hesitated to offer me aid and welcome me into his home. I am forever grateful for the love they give me and cannot image a life without it.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: LITERATURE REVIEW

1.1 Biology and Ecology of WCR

The western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte, is a univoltine, protandrous insect (Ball 1957, Chiang 1973, Branson 1987, Darnell et al. 2000, Meinke et al. 2009, Spencer et al. 2009). Beetles are 0.6 cm in length and yellowish with two black stripes on the elytra of variable width (Tate and Bare 1946). Eggs are oviposited from July through September and overwinter in the soil until the following season to hatch during late May through early July (Ball 1957, Shaw et al. 1978, Levine and Oloumi-Sadeghi 1991, Gray et al. 1992, Meinke et al. 2009). When overwintering, eggs in the soil can be exposed to extreme environmental conditions and experience high mortality (Gustin 1981, Godfrey et al. 1995). Egg overwintering consists of obligate diapause, a period of suspended development, and facultative post-diapause quiescence/development, triggered by environmental factors (e.g. soil temperature, water content, etc.) (Krysan 1977, 1978, Schaafsma et al. 1991, Meinke et al. 2009). The post-diapause developmental threshold is >11°C soil temperature (Wilde 1971, Krysan 1978, Gustin 1981, Schaafsma et al. 1991, Levine et al. 1992). Diapause duration is genetically based which decreases in duration from south to north (Wilde et al. 1972, Krysan et al. 1977, 1978, Schaafsma et al. 1991). Extended diapause, overwintering for two winters, has been documented to occur to 0.14-0.24% of WCR eggs (Levine and Oloumi-Sadeghi 1992).

The WCR larval stage consists of three instars and the rate of development is temperature dependent (Kuhlman et al. 1970, Fisher 1986, Jackson and Elliott 1988). After hatching, larvae will begin feeding immediately on suitable host roots, and failure to locate a root system within 24hrs dramatically decreases their survival rate (Branson 1989, Clark et al. 2006, Spencer et al. 2009). Larvae can only survive on a number of grass species with maize serving as the major host in modern agroecosystems (Branson and Ortman 1967, 1970, Clark and Hibbard 2004, Oyediran et al 2004). Locating root systems depends on the volatile cues released by roots such as carbon dioxide and 6-methoxy-2-benzoxazolinone (MBOA), a semiochemical which is released by maize and multiple grass host species (Bjostad and Hibbard 1992). Male larvae and pupae develop faster than females at a temperature range of 19-30°C but both sexes have a lower threshold development near 9°C. Temperatures greater than 30°C decrease survival and increases deformities, (Jackson and Elliott 1988).

As larval densities increase, adult emergence decreases (Chiang et al. 1980). Small-moderate larval densities with lightly-damage root systems give rise to 1:1 (male: female) adult sex ratio (Weiss et al. 1985). High rootworm larval densities cause severe root damage and often results in altered sex ratio (e.g., 2.4 male:1 female) as more males' complete development first on the root system before great damage is done (Weiss et al. 1985).

Adult emergence begins during late June through early July with peak emergence during the last half of July (Quiring and Timmins 1990, Darnell et al, 2000). In continuous maize (≥ 2 successive years of cultivation), protandry in this species leads to peak cumulative male emergence occurring earlier than peak female cumulative emergence resulting in male bias early in the season shifting more toward female bias later in the season (Short and Hill 1972). This provides time for adult males to sexually mature after emergence and then immediately mate with adult females which are sexually mature upon emergence (Ball, 1957, Branson 1987, Hammack 1995, Meinke et al. 2009, Spencer et al. 2009). Adults feed on pollen but also other reproductive parts of plants (Ball 1957, Moeser and Hibbard 2005, Campbell and Meinke 2006). Total adult emergence can be affected by soil insecticide applications and transgenic maize, directly or indirectly affecting the developmental time of larvae, and delay adult emergence (Sutter at al. 1991, Becker 2006, Meinke et al. 2009, Petzold-Maxwell et al. 2013). As density of larvae increases, and food becomes limiting, development time of the larval stages increases and subsequent adult size decreases (Branson and Sutter 1985, Weiss et al. 1985, Elliot et al. 1989, Onstad et al. 2006).

1.2. WCR as a pest insect

The WCR is one of the most economically important insect pests of field maize (*Zea mays* L.) in the United States Corn Belt. In Nebraska and the western Corn Belt, the demand for maize is high for confined livestock operations and ethanol production, leading to higher adoption of continuous maize (Souza et al. 2019). This can lead to build-up of WCR densities (Hill and Mayo 1980, Meinke et al. 2009), making the annual management of this pest a considerable challenge. The yield loss from WCR damage and control costs can exceed $2 billion dollars annually (Wechsler and Smith 2018). The 2nd and 3rd larval instars cause the majority of root feeding damage and may cause significant yield loss to maize (Spike and Tollefson 1991, Levine and Oloumi-Sadeghi 1992, Urías-López and Meinke 2001). Larval injury to maize roots may decrease the plant's ability to obtain nutrients and water, thus reducing plant growth, stability, and yield (Kahler et al. 1985, Spike and Tollefson 1991, Godfrey et al. 1993, Hou et al. 1997, Urías-López et al. 2000, Urías-López and Meinke 2001 Tinsley et al. 2013). Following the node-injury scale developed by Oleson et al. (2005), each composite node of root injury can result in a yield loss of 15-17% (Dun et al. 2010, Tinsley et al. 2013). When high adult densities are present, silk clipping damage can occur which can reduce pollination and fertilization of maize resulting in loss of kernels and reduced grain yield (Chiang 1973, Culy et al. 1992; Meinke 2014).

1.3. WCR Management Tactics and Adaptation to Selection Pressure

1.3.1. Crop Rotation

Crop rotation is a cultural agricultural practice to manage western corn rootworm that involves the annual rotation of field maize to a non-host plant, like soybeans (*Glycine max.* L.). Crop rotation provides excellent control of WCR larvae without the need for other chemical control tactics. The maize-soybean rotation also yields 5-20% more maize than continuous maize production, although yield from non-host plant often provides poorer economic return (Bullock 1992).

Crop rotation was successful in managing WCR for 90 years until 1987 when control failure to rotated maize was observed in Illinois (Gillette 1912, Levine and Oloumi-Sadeghi 1996, Levine et al. 2002). WCR females adapted to crop rotation by exhibiting less affinity for corn as an ovipositional site and depositing eggs in both maize and other surrounding crops, overcoming the rotation effect (Levine et al. 2002, Rodon and Gray 2004). Crop rotation then became an ineffective management tactic in some areas of the eastern Corn Belt due to this WCR behavior shift (Spencer et al. 1997, Levine et al. 2002, Gray et al. 2009).

1.3.2. WCR Rotation Resistance

The rotation resistant WCR strain has spread to parts of Illinois, Indiana, Wisconsin, Michigan, Ohio, eastern Iowa, Missouri, and the Canadian province, Ontario (Levine et al. 2002, Meloche and Hermans 2004, Gray et al. 2009, Dunbar and Gassmann 2013). In areas where crop rotation is common, Onstad et al. (2001b) suggested that a landscape composed of ≥80% annual crop rotation is necessary for selection of phenotypes which explore new hosts. This was reflected in a study in Illinois were rotation resistant WCR eggs were recovered from not only maize and soybean, but oats (*Avena sativa* L.), and alfalfa (*Medicago sativa* L.) confirming that relaxed oviposition preference is a factor in crop rotation resistance (Rondon and Gray 2004, Pierce and Gray 2006, Spencer et al. 2009).

In crop-rotation fields, selection pressure is placed on the WCR population because only WCR oviposited in soybeans the previous season survive, and larvae in maize do not survive the soybean rotation (Dunbar and Gassmann 2013). The beetles can feed on soybean tissue but the poor nutrition from a soybean diet causes dietary stress that increases oviposition rate and mobility between soybean and maize fields (Mabry and Spencer 2003, 2004, Spencer et al. 2005). Only 20% of females found in soybean fields are gravid, requiring movement back to maize fields to feed and develop egg clutches (Mabry and Spencer 2003). This movement activity, combined with the effect of soybean tissue promoting oviposition in rotation resistant females, may provide a mechanism behind rotation resistance WCR movement (Mabry et al. 2004, Spencer et al. 2005, Spencer et al. 2014).

Recently, larval gut bacteria were associated with the adaptation of WCR to crop rotation by facilitating soybean herbivory (Chu et al. 2013). Comparison between rotation resistant WCR gut microbiota and wild-type WCR revealed shifts in the bacterial community structure when introduced to soybean tissue diet. Antibiotic treatment that depressed WCR gut bacteria on rotation resistant WCR revealed reduced tolerance to soybean tissue to wild-type levels (Chu et al. 2013). Thus, selection of WCR gut bacteria by maize-soybean crop rotation contributes to rotation resistant WCR.

1.3.3. WCR Soil Insecticide and Resistance

Soil insecticides were first introduced in the 1940s with the broadcast application of a chlorinated hydrocarbon, benzene hexachloride (BHC), and later cyclodiene insecticides for larval control (Hill et al. 1948, Muma et al. 1949, Cox and Lilly 1953, Ball and Hill 1953). Widespread WCR resistance to cyclodienes and BHC occurred within a decade of use (Roselle et al. 1959, 1960, 1961, Ball and Weekman 1962, Ball 1983); resistance and the persistence of these insecticides in the soil caused them to be banned in 1972 by the EPA (EPA 1998). More than 50 years since the introduction, organochlorine resistance alleles are still present within present WCR populations (Parimi et al. 2006, Pereira et al. 2017).

Organophosphate (OP) and carbamate soil insecticides filled the void left from the ban on organochlorines and resistance to cyclodienes (Ball 1969, Meinke et al. 1998). Because they were expensive, they were only applied in-furrow or as bands across a row which created a natural untreated refuge between rows. This built-in refuge is likely the reason no WCR resistance has occurred from OP and carbamate soil insecticides compared to cyclodiene broadcast soil applications (Gray et al. 1992, Parimi et al. 2006, Souza et al. 2020).

1.3.4. Foliar Insecticide

Foliar insecticides target adult WCR to protect maize silk from silk feeding, allowing pollination, or reducing WCR female density and oviposition in maize fields, thereby scaling down larval injury the following season (Meinke et al. 2021). After WCR adapted to cyclodiene soil insecticides, use of organophosphate and carbamate foliar insecticides was adopted as an alternative WCR management strategy in parts of the western Corn Belt (Mayo 1976, Pruess et al. 1974). Foliar insecticide application provided excellent protection of aboveground maize plant parts and indirectly protected the root system by reducing oviposition and larval densities the following season if properly timed with gravid female emergence (Mayo 1976, Levine and Oloumi-Sadeghi 1991, Zhu et al. 2005). However, due to selection pressure from broadcast applications, anecdotal reports of reduced control with foliar-applied insecticide became more common (Meinke et al. 1998, Pereia et al. 2015).

1.3.5. Foliar Insecticide Resistance

As a stand-alone WCR adult management tactic, aerial insecticide applications after long-term, repeated use selected for resistance evolution. Multiple broadcast applications of foliar insecticide within and among years resulted in WCR field-evolved resistance to methyl-parathion and carbaryl (Meinke et al. 1998, Scharf et al. 1999, Wright et al 2000, Zhu et al. 2005). Topical bioassays of WCR populations in Nebraska documented 16- and 9-fold resistance levels to methyl-parathion and carbaryl, respectively (Meinke et al. 1998, Meinke et al. 2021). In absence of selection pressure, WCR populations from Nebraska were shown to retain resistance to methyl-parathion, up to 19-fold, after 5-6 generations (Parimi et al. 2006). During the 1990s, regulatory action removed many of the organophosphate and carbamate products from the market including formulations of methyl parathion needed to provide adequate residual activity in the field. Therefore, adult WCR management as a stand-alone tactic was abandoned in the western Corn Belt and foliar applications are now primarily used to complement other tactics (Meinke et al. 2021).

Currently, only pyrethroid insecticides (permethrin, bifenthrin, etc.) a few organophosphate insecticides (chlorpyrifos, dimethoate, etc.) and an oxadiazine class insecticide, indoxacarb, are available for adult WCR control (Elliot et al. 1978, van Rozen and Ester 2010, Souza et al. 2019). These compounds are used in conjunction with the primary tactic, transgenic maize, to manage densities or to help mitigate evolving resistance to transgenic traits (Souza et al. 2019). Pyrethroids and OPs are also used to manage western bean cutworm (*Striacosta albicosta* Smith), two-spotted spider mite (*Tetranychus urticae* Koch), and Banks grass mite (*Oligonychus pratensis* Banks) in the same continuous maize systems as WCR (Meinke et al. 2021). The nontarget and target insecticide exposure to WCR places significant selection pressure on WCR over time which facilitated recent field-evolved resistance to bifenthrin in southwestern areas of Nebraska and Kansas (Pereira et al. 2015, Souza et al. 2019). Dimethoate (OP) laboratory assays likewise documented low levels of WCR resistance in a WCR population from southwestern Nebraska, nevertheless, this did not significantly affect control when label rates of formulated product were bioassayed in simulated aerial applications (Souza et al. 2019).

1.3.6. Bt Maize

Transgenic maize expressing insecticidal protein(s) derived from *Bacillus thuringiensis* (Bt) Berliner, a gram-positive soil-dwelling bacterium, is a genetically modified crop used to control WCR larvae. Before the development of Bt transgenic hybrids, Bt has been used as a biopesticide for decades (Bravo et al. 2017). Currently four Bt Cry toxins, Cry3Bb1, Cry34/35Ab1, mCry3A, and eCry3.1Ab have been commercialized either as single-traits or in pyramids (Wangila et al. 2015, Gassmann et al 2020). The rapid adoption of Bt maize by growers in the U.S. Corn Belt comes from three benefits: 1) protection from insect damage thereby increasing yield, 2) other insecticidal products are reduced, and 3) ease of use (Head and Ward 2009). Today, many Bt strains and Bt crops are used worldwide with selectivity against various pest species in Lepidoptera, Diptera, Coleoptera, etc., with no toxicity to human health (Schnepf et al. 1998, Mendelsohn et al. 2003, James 2010, Bravo et al. 2011, 2013).

The insecticidal properties of Bt maize are from the δ-endotoxins in the Cry Family that are specific to Coleoptera, Hymenoptera, Lepidoptera, and Diptera (de Maagd et al. 2001, Pardo-Lopez et al. 2013). The Cry toxin has three structural domains called Domain I, Domain, II, and Domain III (Pardo-López et al. 2013, Osman et al. 2015). Domain I is composed of seven alpha-helices that functions as an ion channel that inserts the toxin on contact with epithelium cells (Falnes and Sandvig 2000). Domain II consists of antiparallel beta-sheets and Domain III a sandwich of two antiparallel beta-sheets, both domains are responsible for receptor and host specificity (de Maagd et al. 1999, Pardo-Lopez et al. 2013). The δ-endotoxins need to be ingested and proteolytically processed in the high pH found in the midgut to produce a toxic effect (Osman et al. 2015). In the midgut, the protoxin is cleaved by proteases and forms 20-60 active toxin fragments, depending on the toxin, which bind to midgut receptors (de Maagd et al. 2001, Pardo-López et al. 2013). Insertion of toxins into the epithelium midgut cells causes cell lysis and osmotic shock from influx of solutes, leading to cell death (Knowles and Ellar 1987, Gill et al. 1992, Pardo-López et al. 2013).

1.3.7. High-dose/Refuge Strategy

A refuge strategy accompanies the planting of Bt maize to produce insects that are susceptible to the Bt toxin, preserving the susceptible alleles and increasing the number of susceptible individuals within a population (Tabashnik et al. 2003). Combined with a high dose of toxin expressed by the Bt plant, the high-dose/refuge strategy is an IRM program to slow field-evolved resistance to Bt toxins and extend trait durability (Tabashnik et al. 2003). This IRM strategy is required by the U.S. Environmental Protection Agency when Bt traits are deployed in the field (EPA 2021). Single-trait Bt maize deployment requires a 20% refuge and a high dose (25 times amount to kill > 99%) (EPA 1998, Gould 1998, Ives and Andow 2002, Storer 2003, Devos et al. 2013). For pyramided hybrids, with multiple modes of action, the refuge percentage requirement is lower (5-10%) (DiFonzo and Porter 2021). When the conditions of the high-dose/refuge strategy are met, Bt maize is able to achieve sustained insecticidal effect and pest susceptibility (Hutchison et al. 2010, Tabashnik et al. 2010, Tabashnik and Gould 2012).

1.3.8. WCR Bt Resistance

The currently registered WCR-active Cry traits violate several key assumptions of the high dose/refuge strategy, namely, plants express Bt toxins that meet the high-dose definition, initial resistance allele frequencies are rare, inheritance is recessive, and susceptible adults from nearby refuges will disperse far and mate with nearly all rare resistant individuals that emerge from Bt fields (Andow et al. 2016). All currently registered WCR-active traits are less than high dose and naturally produce some survival to the adult stage. Because of Cry traits not meeting high-dose/refuge strategy criteria and the overuse of single-protein Cry3Bb1, mCry3A, and/or Cry34/35Ab1, rapid field-evolved resistance has appeared in the U.S. Corn Belt (Gassmann et al. 2011, Wangila et al. 2015, Gassmann 2021). Additional factors that have contributed to WCR resistance evolution to Bt traits include: grower noncompliance with the refuge strategy, minimal fitness costs of resistance, and nonrandom mating due to non-synchronous adult emergence, (Devos et al. 2013, Andow et al. 2016, Gassmann et al. 2021).

Field and laboratory selection experiments have documented evolution of resistance to Cry3Bb1, Cry34/35Ab1, and mCry3A when selection pressure is maintained for $\geq$ 3 generations (Lefko et al. 2008, Meihls et al. 2008, Oswald et al. 2011, Frank et al. 2013, Gassmann et al. 2014). Larval bioassays of field populations confirmed resistance to Bt-traits in areas of Iowa, Nebraska, Illinois, and Minnesota (Gassmann et al. 2011, 2020, Wangila et al. 2015, Ludwick et al. 2017). Cross-resistance has been documented between Cry3 Bt traits, but the level of cross-resistance has been variable (Gassmann et al. 2011, Wangila et al. 2015, Reinders et al. 2018, Zukoff et al. 2016). This greatly reduces the ability of growers to rotate traits in current management programs. There is no cross resistance between Cry3 traits and Cry34/35Ab1 (Gassmann et al. 2011, Wangila et al. 2015). Current WCR pyramids contain one or two traits that were previously sold as single traits which potentially compromises efficacy of pyramided hybrids in areas where resistance occurs. Current evidence suggests WCR resistance-evolution events in maize fields are independently driven by local management practices and local gene flow among fields (Reinders et al. 2018, Gassmann 2021).

1.4. Sampling Technique

1.4.1. Whole-Plant Count

Whole-plant count (WPC) sampling of western corn rootworm is a visual count estimate of beetle populations used to make rootworm management decisions (Darnell et al. 1999). The WPC method is ideal for quick, on the spot, population estimates; consequently, sex ratios cannot be determined with this method. The sampling technique involves an examination of a maize plant for all WCR beetles, top-to-bottom, each leaf surface and ear (Darnell et al. 1999). Each WPC is taken >30-40 m apart from each other to be spatially independent (Hein and Tollefson 1984, 1985a; Darnell et al. 1999). The WCR economic threshold indices for WPC vary regionally but are higher in continuous corn than first -year corn due to the greater proportion of females often found in first-year corn (Godfrey and Turpin 1983, Meinke 1995, Wright et al 1999)

1.4.2. Unbaited Pherocon® AM Trap

Use of Yellow unbaited Pherocon AM traps in a WCR sampling program involves attaching a sticky, rectangular cardboard trap around maize plants (ear height), then leaving the trap for a period of time to estimate WCR population density (Hein and Tollefson 1984, 1985a; Karr and Tollefson 1987; O’Neal et al. 2001; Pierce and Gray 2007). Trap sampling is recommended due to ease of use, cheap cost, and compact shape (Hein and Tollefson 1984). Traps are placed on each plant >30 m apart from each other for spatially independent samples (Midgarden et al. 1993). The trap economic threshold ranges from 25-40 beetles/trap/week or 4-6 beetles/trap/day, depending on field conditions; higher economic thresholds are needed for continuous maize fields (Hein and Tollefson 1985a, Edwards et al. 1994, Wilde 1999). For a standard error of <10%, 12 traps should be deployed per field (Hein and Tollefson 1985a).

The WCR density and sex ratio varies depending on monitoring technique and season. Short and Hill (1972) documented sex ratios shift from male bias to female bias seasonally with 10-min. beetle collections. Godfrey and Turpin (1983) reported yellow sticky traps caught a higher total female beetle density in continuous maize than first-year maize early in the emergence period but a reverse of this trend near the end of the season. Difference in capture density also arises from material used in the sampling technique. For example, Kuhar and Youngman (1995) observed Olson traps captured significantly more WCR adults than modified Pherocon AM trap primarily due to trap color differences. WCR sex ratio and density can be biased by the sampling technique, trap location, and timing of sampling, so sampling data needs to relate to actual density and sex ratios in the field to be useful in management programs (Kuhar and Youngman 1995, Campbell and Meinke 2006, Meinke et al. 2009).

1.4.3. Emergence Cage

Emergence cage traps consist of a wooden frame and screen/mesh attached and are used to estimate absolute adult emergence from a field. Emergence cages can either require a cut maize plant or modified to allow for plant growth through the center of the trap (Fisher et al. 1980, Hein et al. 1985b, Pierce and Gray 2007). WCR beetles are captured in a glass jar placed at the top of each cage to be changed regularly (Pierce and

Gray 2007). The sample size needed for a standard error <10% is 80 quadrats, one cage per quadrant (Hein and Tollefson 1985b).

1.5. Justification for Research

The WCR is a very adaptive insect species that has evolved resistance to rootworm management tactics to varying degrees. It has adapted to crop rotation, soil and foliar insecticides, and single-trait transgenic maize which leaves pyramided traits and few insecticides available for continued rootworm management in some locations. The reduced susceptibility to single-trait transgenic maize is especially concerning as it can accelerate field-evolved resistance to pyramided maize that contains a trait a WCR population was previously exposed to or is resistant to. Cross-resistance between Cry3Bb1 and mCry3A was documented which also reduces opportunities to rotate traits in management programs. It is important to revisit existing tactics and determine the best ways to more holistically utilize these within an integrated framework to manage WCR. The integration of foliar insecticides with pyramids to both control WCR densities and reduce selection pressure in areas with evolving resistance to Bt traits could be an important component of management programs in continuous maize. However, the efficacy of formulated foliar insecticide on field populations and their longer-term effects are not well documented. Field-evolved resistance of WCR has also been confirmed to pyrethroids in areas of southwestern Nebraska and Kansas with the current susceptibility level in eastern Nebraska unknown. There is a need to fully understand the current level of WCR susceptibility to insecticides used to target adults in eastern Nebraska and the possible longer-term benefits of adult control on population densities to develop effective IPM/IRM programs without increasing the potential for resistance evolution.

Therefore, this study aims to investigate the current susceptibility of WCR field populations in northeast Nebraska to provide critical information for future management programs. The research project has four objectives:

1. Evaluate the within season efficacy of formulated foliar insecticides on adult WCR populations in northeast Nebraska (Chapter 2).
2. Determine the potential effect of adult foliar insecticide application on WCR population density the following season (Chapter 2).
3. Determine the relationship between whole plant count and unbaited sticky trap sampling techniques when predicting WCR density (Chapter 2).
4. Characterize the susceptibility of WCR populations in northeast Nebraska to active ingredient insecticides, bifenthrin, chlorpyrifos, and dimethoate (Chapter 3).

1.6. Literature Cited

Abou-Assaf, N., J. R. Coats, M. E. Gray, and J. J. Tollefson. **1986**. Degradation of isofenphos in cornfields with conservation tillage practices. J. Environ. Sci. Health B. 21: 425–446.

Andow, D. A., S. G. Pueppke, A. W. Schaafsma, A. J. Gassmann, T. W. Sappington, L. J. Meinke, P. D. Mitchell, T. M. Hurley, R. L. Hellmich, and R. P. Porter. **2016**. Early detection and mitigation of resistance to Bt maize by western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 109: 1–12.

Ball, H. J. 1957. On the biology and egg-laying habits of the western corn rootworm. J. Econ. Entomol. 50: 126–128.

Ball, H. J., and R. E. Hill. 1953. You can control corn rootworm. Neb. Exp. Stn. Quart. 1: 3–4.

Ball, H. J., and R. E. Roselle. 1954. You can control corn rootworms. University Nebraska College of Agricultural Extension Circular; University of Nebraska-Lincoln: Lincoln, NE, USA.

Becker, S. C. 2006. Stage-specific development and mortality of western and norther corn rootworm reared on transgenic event MON863 and on a non-transgenic isoline field corn hybrid. M.S. Thesis, University of Nebraska, Lincoln.

Bjostad, L. B., and B. E. Hibbard. **1992**. 6-Methoxy-2-benzoxazolinone: A semiochemical for host location by western corn rootworm larvae. J. Chem. Ecol. 18: 931–944.

Blandino, M., C. Ferracini, I. Rigamonti, G. Testa, M. A. Saladini, C. Jucker, M. Agosti, A. Alma, and A. Reyneri. **2017**. Control of western corn rootworm damage by application of soil insecticides at different maize planting times. Crop Protect. 93: 19–27.

Branson, T. F. 1987. The contribution of prehatch and posthatch development to protandry in the chrysomelid, *Diabrotica virgifera virgifera*. Entomol. Exp. Appl. 43: 205–208.

Branson, T. F., 1989. Survival of starved neonate larvae of *D. virgifera virgifera* LeConte (Coleoptera: Chrysomelidae). J. Kansas Entomol. Soc. 62: 521–523.

Branson, T. F., and E. E. Ortman. 1967. Fertility of western corn rootworm reared as larvae on alternate hosts. J. Econ. Entomol. 60: 595.

Branson, T. F., and E. E. Ortman. **1970**. The host range of larvae of the western corn rootworm: Further Studies. J. Econ. Entomol. 63: 800–803.

Branson, T. F., and G. R. Sutter. 1985. Influence of population density of immatures on size, longevity, and fecundity of adult *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). Environ. Entomol. 14(6): 687-690.

Bravo, A. S., I. Gómez, H. Porta, B. I. García-Gómez, C. Rodriguez-Almazan, L. Pardo, and M. Soberón. 2013. Evolution of *Bacillus thuringiensis* Cry toxins insecticidal activity. Microb. Biotechnol. 6: 17-26.

Bravo, A., S. Likitvivatanavong, S. S. Gill, and M. Soberón. **2011**. *Bacillus thuringiensis*: a story of a successful bioinsecticide. Insect Biochem. Mol. Biol. 41: 423–431.

Bravo, A., S. Pacheco, I. Gómez, B. Garcia-Gómez, J. Onofre, and M. Soberón. 2017. Insecticidal proteins from *Bacillus thuringiensis* and their mechanism of action, pp. 53-66. *In* L. M. Fiuza, R. A. Polanczyk, and N. Crickmore (eds.), *Bacillus thuringiensis* and *Lysinibacillus spahericus*. Springer, Switzerland.

Bullock, D. G. 1992. Crop rotation. CRC Crit. Rev. Plant Sci. 11: 309–309.

Calvin, D. D., L. Smith, and P. R. Rebarchak. **2004a**. Corn rootworm larval control, 1999. Arthropod Manag. Tests. 29. doi.org/10.1093/amt/29.1.F17

Calvin, D. D., S. Sked, and S. Smiles. **2004b**. Corn rootworm larval control, 2002. Arthropod Manag. Tests. 29. doi.org/10.1093/amt/29.1.F19.

Campbell, L. A., and L. J. Meinke. 2006. Seasonality and adult habitat use by four *Diabrotica* species at prairie-corn interfaces. Environ. Entomol. 35: 922-936.

Chandler, L. D. 2003. Corn rootworm areawide management program: United States Department of Agriculture-Agricultural Research Service. Pest Manag. Sci. 59: 605–608.

Chiang, H. C. 1973. Bionomics of the northern and western corn rootworms. Annu. Rev. Entomol. 18: 47–72.

Chiang, H. C., L. K. French, and D. E. Rasmussen. **1980**. Quantitative relationship between western corn rootworm population and corn yield. J. Econ. Entomol. 73: 665–666.

Chu, C.-C., J. L. Spencer, M. J. Curzi, J. A. Zavala, and M. J. Seufferheld. **2013**. Gut bacteria facilitate adaptation to crop rotation in the western corn rootworm. Proc. Natl. Acad. Sci. 110: 11917–11922.

Clark, T. L., and B. E. Hibbard. **2004**. Comparison of nonmaize hosts to support western Corn rootworm (Coleoptera: Chrysomelidae) larval biology. Environ. Entomol. 33: 681–689.

Clark, P.L., T. T. Vaughn, L. J. Meinke, J. Molina-Ochoa, and J. E. Foster. 2006. *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) larval feeding behavior on transgenic maize (MON 863) and its isoline. J. Econ. Entomol. 99: 722–727.

Cox, C. H., J. H. Lilly. 1953. Chemical control of the corn rootworm. *J. Econ. Entomol.* 46: 217–224.

Dana, L. A., T. E. Hunt, and K. Jarvi. **2013**. Corn rootworm larval control in corn, 2012. Arthropod Manag. Tests. 38. doi.org/10.4182/amt.2013.F8.

Darnell, S. J., L. J. Meinke, and L. J. Young. 2000. Influence of corn phenology on adult western corn rootworm (Coleoptera: Chrysomelidae) distribution. Environ. Entomol. 29: 587-595.

Darnell, S. J., L. J. Meinke, L. J. Young, and C. A. Gotway. **1999**. Geostatistical investigation of the small-scale spatial variation of western corn rootworm (Coleoptera: Chrysomelidae) adults. Environ. Entomol. 28: 266–274.

de Maagd, R. 2001. How *Bacillus thuringiensis* has evolved specific toxins to colonize the insect world. Trends Genet. 17: 193–199.

de Maagd, R. A., D. Bosch, and W. Stiekema. **1999**. *Bacillus thuringiensis* toxin-mediated insect resistance in plants. Trends Plant Sci. 4: 9–13.

Devos, Y., L. N. Meihls, J. Kiss, and B. E. Hibbard. **2013**. Resistance evolution to the first generation of genetically modified *Diabrotica*-active Bt-maize events by western corn rootworm: management and monitoring considerations. Transgenic Res. 22: 269–299.

DeVries, T., and R. J. Wright. **2002a**. Larval corn rootworm control, 2001A. Arthropod Manag. Tests. 27. doi.org/10.1093/amt/27.1.F15.

DeVries, T., R. J. Wright, L. Meinke, and B. Siegfried. **2002b**. Evaluation of soil insecticides for control of resistant corn rootworm, 2001. Arthropod Manag. Tests. 27. doi.org/10.1093/amt/27.1.F18.

DiFonzo, C. 2021. The handy Bt trait table for U.S. corn production, Agrilife.org. Pat Porter/Texas A&M University. https://www.texasinsects.org/bt-corn-trait-table.html

Dunbar, M. W., and A. J. Gassmann. **2013**. Abundance and distribution of western and northern corn rootworm (*Diabrotica* spp.) and prevalence of rotation resistance in eastern Iowa. J. Econ. Entomol. 106: 168–180.

Edwards, C. R., W. B. Larry, and F. T. Turpin. 1994. Field crop insects. Managing corn rootworms 1994. Purdue University, Cooperative Extension Service, E-49, 1–6, West Lafayette, IN, USA.

Elliott, N. C., G. R. Sutter, T. F. Branson, and J. R. Fisher. **1989**. Effect of population density of immatures on survival and development of the western corn rootworm (Coleoptera: Chrysomelidae). J. Entomol. Sci. 24: 209–213.

EPA, 1998. Final report of the FIFRA Scientific Advisory Panel Subpanel on *Bacillus thuringiensis* (Bt) plant-pesticides and resistance management. <http://www.epa.gov/scipoly/sap/meetings/1998/february/finalfeb.pdf>.

(EPA) EPA. **2021**. EPA. (https://www.epa.gov/ingredients-used-pesticide-products/ddt-brief-history-and-status). (accessed 5 July, 2021).

(EPA) EPA. **2021**. EPA. (https://www.epa.gov/regulation-biotechnology-under-tsca-and-fifra/insect-resistance-management-bt-plant-incorporated).

Falnes, P. O., and K. Sandvig. 2000. Penetration of protein toxins into cells. Curr. Opin. Cell Biol. 12: 407-413.

Felsot, A. S. 1989. Enhanced biodegradation of insecticides in soil: implications for agroecosystems. Annu. Rev. Entomol. 34: 453–476.

Finney, D. J. 1971. Probit analysis, 3rd ed. Cambridge University Press, Cambridge.

Fisher, J. R., J. J. Jackson, G. R. Sutter, and T. F. Branson. **1986**. Influence of adult diet on damage to corn by larvae of the western corn rootworm *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). J. Econ. Entomol. 79: 114–115.

Frank, D. L., A. Zukoff, J. Barry, M. L. Higdon, and B. E. Hibbard. **2013**. Development of resistance to eCry3.1Ab-expressing transgenic maize in a laboratory-selected population of western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 106: 2506–2513.

Furlan, L., S. Canzi, A. Di Bernardo, and C. R. Edwards. **2006**. The ineffectiveness of insecticide seed coatings and planting-time soil insecticides as *Diabrotica virgifera virgifera* LeConte population suppressors. J. Appl. Entomol. 130: 485–490.

Gassmann, A. J. 2021. Resistance to Bt maize by western corn rootworm: effects of pest biology, the pest–crop interaction and the agricultural landscape on resistance. Insects. 12: 136.

Gassmann, A. J., J. L. Petzold-Maxwell, E. H. Clifton, M. W. Dunbar, A. M. Hoffmann, D. A. Ingber, and R. S. Keweshan. 2014. Field-evolved resistance by western corn rootworm to multiple *Bacillus thuringiensis* toxins in transgenic maize. Proc. Nat. Acad. Sci. USA 111: 5141-5146.

Gassmann, A. J., J. L. Petzold-Maxwell, R. S. Keweshan, and M. W. Dunbar. 2011. Field-evolved resistance to Bt maize by western corn rootworm. PLoS ONE 6: e22629.

Gassmann, A. J., R. B. Shrestha, A. L. Kropf, C. R. St Clair, and B. D. Brenizer. **2020**. Field-evolved resistance by western corn rootworm to Cry34/35Ab1 and other *Bacillus thuringiensis* traits in transgenic maize. Pest Manage. Sci. 76: 268–276.

Gassmann, A. J., R. B. Shrestha, S. R. K. Jakka, M. W. Dunbar, E. H. Clifton, A. R. Paolino, D. A. Ingber, B. W. French, K. E. Masloski, J. W. Dounda, and C. R. St. Clair. 2016. Evidence of resistance to Cry34/35Ab1 corn by western corn rootworm (Coleoptera: Chrysomelidae): root injury in the field and larval survival in plant-based bioassays. J. Econ. Entomol. 109: 1872-1880.

Gill, S. S., E. A. Cowles, and P. V. Pietrantonio. 1992. The mode of action of *Bacillus thuringiensis* endotoxins. Annu. Rev. Entomol. 37: 615-636.

Godfrey, L. D., and F. T. Turpin. 1983. Comparison of western corn rootworm (Coleoptera: Chrysomelidae) adult populations and economic thresholds in first-year and continuous corn fields. J. Econ. Entomol. 76: 1028-1032.

Godfrey, L. D., L. J. Meinke, and R. J. Wright. 1993. Vegetative and reproductive biomass accumulation in field corn: response to root injury by western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 86: 1557-1573.

Gorder, G. W., P. A. Dahm, and J. J. Tollefson. **1982**. Carbofuran persistence in cornfield soils. J. Econ. Entomol. 75: 637–642.

Gray, M. E., A. S. Felsot, K. L. Steffey, and E. Levine. **1992**. Planting time application of soil insecticides and western corn rootworm (Coleoptera: Chrysomelidae) emergence: implications for long-term management programs. J. Econ. Entomol. 85: 544–553.

Gray, M. E., T. W. Sappington, N. J. Miller, J. Moeser, and M. O. Bohn. 2009. Adaptation and invasiveness of western corn rootworm: intensifying research on a worsening pest. Annu. Rev. Entomol. 54: 303-321.

Godfrey, L. D., and F. T. Turpin. **1983**. Comparison of western corn rootworm (Coleoptera: Chrysomelidae) adult populations and economic thresholds in first-year and continuous corn fields. J. Econ. Entomol. 76: 1028–1032.

Godfrey, L. D., L. J. Meinke, R. J. Wright, and G. L. Hein. 1995. Environmental and edaphic effects on western corn rootworm (Coleoptera: Chrysomelidae) overwintering egg survival. J. Econ. Entomol. 88: 1445-1454.

Gould, F. 1998. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. Ann. Rev. Entomol. 43:701–726.

Gustin, R. D. 1981. Soil temperature environment of overwintering western corn rootworm eggs. Environ. Entomol. 10(4): 483-487.

Hamilton, E. W. 1965. Aldrin resistance in corn rootworm beetles. J. Econ. Entomol. 58: 296–300.

Hammack, L. 1995. Calling behavior in female western corn rootworm beetles (coleoptera: Chrysomelidae). Ann. Entomol. Soc. Am. 88: 562–569.

Harris, C. R., R. A. Chapman, C. Harris, and C. M. Tu. **1984**. Biodegradation of pesticides in soil: Rapid induction of carbamate degrading factors after carbofuran treatment. J. Environ. Sci. Health B. 19: 1–11.

Head, G., and D. Ward. **2009**. Insect resistance in corn through biotechnology, pp. 31–40. *In* Molecular Genetic Approaches to Maize Improvement. Springer, Berlin.

Hellmich, R. L., and K. A. Hellmich. 2012. Use and impact of Bt maize. Nat. Educ. Knowl. 3: 4.

Hill, R. E., and Z B Mayo. 1980. Distribution and abundance of corn rootworm species as influenced by topography and crop rotation in eastern Nebraska. Environ. Entomol. 9: 122-127.

Hill, R. E., E. Hixson, and M. H. Muma. **1948**. Corn rootworm control tests with Benzene Hexachloride DDT, nitrogen fertilizers and crop rotations. J. Econ. Entomol. 41: 392–401.

Hills, T. M., and D. C. Peters. **1972**. Methods of applying insecticides for controlling western corn rootworm larvae. J. Econ. Entomol. 65: 1714–1718.

Hutchison, W. D., E. C. Burkness, P. D. Mitchell, R. D. Moon, T. W. Leslie, S. J. Fleischer, M. Abrahamson, K. L. Hamilton, K. L. Steffey, M. E. Gray, R. L. Hellmich, L. V. Kaster, T. E. Hunt, R. J. Wright, K. Pecinovsky, T. L. Rabaey, B. R. Flood, and E. S. Raun. **2010**. Areawide suppression of European Corn Borer with Bt maize reaps savings to non-Bt maize growers. Sci. 330: 222–225.

Ives, A. R., and D. A. Andow. 2002. Evolution of resistance to Bt crops: directional selection in structured environments. Ecol. Lett. 5:792–801.

Jackson, J. J., and N. C. Elliott. **1988**. Temperature-dependent development of immature stages of the western corn rootworm, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). Environ. Entomol. 17: 166–171.

James, C. 2010. A global overview of biotech (GM) crops: Adoption, impact and future prospects. GM Crops. 1: 8–12.

Kahler, A. L., A. E. Olness, G. R. Sutter, C. D. Dybing, and O. J. Devine. 1985. Root damage by western corn rootworm and nutrient content in maize. Agron. J. 77: 769-774.

Knolhoff, L. M., J. J. Glas, J. L. Spencer, and M. R. Berenbaum. **2010**. Oviposition behaviors in relation to rotation resistance in the western corn rootworm. Environ. Entomol. 39: 1922–1928.

Knowles, B. H., and D. J. Ellar. 1987. Colloid-osmotic lysis is a general feature of the mechanism of action of *Bacillus thuringiensis* d-endotoxins with different insect specificity. Biochim. Biophys. Acta. Gen. Subj. 924(3): 509-518.

Krysan, J. L. 1978. Diapause, quiescence, and moisture in the egg of the western corn rootworm, *Diabrotica virgifera*. J. Insect Physiol. 24(6-7): 535-540.

Krysan, J. L., T. F. Branson, and G. D. Castro. 1977. Diapause in *Diabrotica virgifera* (Coleoptera: Chrysomelidae): a comparison of eggs from temperate and subtropical climates. Entomol. Exp. Appl. 22(1): 81-89.

Kuhlman, D. E., W. L. Howe, and W. H. Luckmann. 1970. Development of immature stages of the western corn rootworm at varied temperatures. Entomol. Soc. Am. N. Cent. Br. Proc. 25: 93-95.

Kuhar, T. P., and R. R. Youngman. **1995**. Sex ratio and sexual dimorphism in western corn rootworm (Coleoptera: Chrysomelidae) adults on yellow sticky traps in corn. Environ. Entomol. 24: 1408–1413.

Lefko, S. A., T. M. Nowatzki, S. D. Thompson, R. R. Binning, M. A. Pascual, M. L. Peters, E. J. Simbro, and B. H. Stanley. **2008**. Characterizing laboratory colonies of western corn rootworm (Coleoptera: Chrysomelidae) selected for survival on maize containing event DAS-59122-7. J. Appl. Entomol. 132: 189–204.

Levine, E., and H. Oloumi-Sadeghi. **1991**. Management of *Diabroticite* Rootworms in corn. Annu. Rev. Entomol. 36: 229–255.

Levine, E., and H. Oloumi-Sadeghi. **1996**. Western corn rootworm (Coleoptera: Chrysomelidae) larval injury to corn grown for seed production following soybeans grown for seed production. J. Econ. Entomol. 89: 1010–1016.

Levine, E., H. Oloumi-Sadeghi, and C. R. Ellis. **1992**. Thermal Requirements, Hatching Patterns, and Prolonged Diapause in Western Corn Rootworm (Coleoptera: Chrysomelidae) Eggs. J. Econ. Entomol. 85: 2425–2432.

Levine, E., J. L. Spencer, S. A. Isard, D. W. Onstad, and M. E. Gray. **2002**. Adaptation of the western corn rootworm to crop rotation: Evolution of a new strain in response to a management practice. Am. Entomol. 48: 94–107.

Ludwick, D. C. 2016. Rootworm management: status of GM traits, insecticides and potential new tools. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources. 11.

Ludwick, D. C., L. N. Meihls, K. R. Ostlie, B. D. Potter, L. French, and B. E. Hibbard. **2017**. Minnesota field population of western corn rootworm (Coleoptera: Chrysomelidae) shows incomplete resistance to Cry34Ab1/Cry35Ab1 and Cry3Bb1. J. Appl. Entomol. 141: 28–40.

Mabry, T. R., and J. L. Spencer. **2003**. Survival and oviposition of a western corn rootworm variant feeding on soybean. Entomol. Exp. Appl. 109: 113–121.

Mabry, T. R., J. L. Spencer, E. Levine, and S. A. Isard. **2004**. Western corn rootworm (Coleoptera: Chrysomelidae) behavior is affected by alternating diets of corn and soybean. Environ. Entomol. 33: 860–871.

Mayo, Z B. 1976. Emergency postplanting applications of insecticides to control larvae of the western and northern corn rootworm in Nebraska. J. Econ. Entomol. 69: 600–602.

Mayo, Z B. 1977. Control of larvae of the western and northern corn rootworm with liquid starter fertilizer-insecticide combinations and the influence of depth of placement. J. Econ. Entomol. 70: 234–236.

Mayo, Z B. 1980. Influence of planting dates on the efficacy of soil insecticides applied to control larvae of the western and northern corn rootworm. J. Econ. Entomol. 73, 211–212.

Meihls, L. N., M. L. Higdon, B. D. Siegfried, N. J. Miller, T. W. Sappington, M. R. Ellersieck, T. A. Spencer, and B. E. Hibbard. 2008. Increased survival of western corn rootworm on transgenic corn within three generations of on-plant greenhouse selection. Proc. Natl. Acad. Sci. USA 105: 19177-19182.

Meinke, L. J. 1995. Adult corn rootworm management. MP63-C, Agricultural Research Division, Institute of Agriculture and Natural Resources. University of Nebraska, Lincoln, Nebraska.

Meinke, L. J., B. D. Siegfried, R. J. Wright, and L. D. Chandler. 1998. Adult susceptibility of Nebraska western corn rootworm (Coleoptera: Chrysomelidae) populations to selected insecticides. J. Econ. Entomol. 91: 594-600.

Meinke, L. J., T. W. Sappington, D. W. Onstad, T. Guillemaud, N. J. Miller, J. Komáromi, N. Levay, L. Furlan, J. Kiss, and F. Toth. 2009. Western corn rootworm (*Diabrotica virgifera virgifera* LeConte) population dynamics. Agr. Forest Entomol. 11: 29-46.

Meinke, L. J., D. Souza, and B. D. Siegfried. 2021. The use of insecticides to manage the western corn rootworm, *Diabrotica virgifera virgifera*, LeConte: history, field-evolved resistance, and associated mechanisms. Insects. 12:112. DOI: 10.3390/insects12020112.

Meloche, F., and P. Hermans. **2004**. Eastward expansion and discovery of the Soybean biotype of western corn rootworm (*Diabrotica virgifera virgifera* LeConte) in Canada. Canadian J. Plant Sci. 84: 305–309.

Mendelsohn, M., J. Kough, Z. Vaituzis, and K. Matthews. **2003**. Are Bt crops safe? Nat. Biotechnol. 21: 1003–1009.

Muma, M. H., R. E. Hill, and E. Hixson. 1949. Soil treatments for corn rootworm control. J. Econ. Entomol. 42: 822-824.

Onstad, D. W. 2006. Modeling larval survival and movement to evaluate seed mixtures of transgenic corn for control of western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 99: 1407–1414.

Onstad, D. W., C. A. Guse, J. L. Spencer, E. Levine, and M. E. Gray. **2001a**. Modeling the dynamics of adaptation to transgenic corn by western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 94: 529–540.

Onstad, D. W., J. L. Spencer, C. A. Guse, E. Levine, and S. A. Isard. **2001b**. Modeling evolution of behavioral resistance by an insect to crop rotation. Entomol. Exp. Appl. 100: 195–201.

Osman, G. E. H., R. Already, A. S. A. Assaeedi, S. R. Organji, D. El-Ghareeb, H. H. Abulreesh, and A. S. Althubiani. 2015. Bioinsecticide *Bacillus thuringiensisa* a comprehensive review. Egypt. J. Biol. Pest. Control. 25: 271-288.

Oswald, K. J., B. W. French, C. Nielson, and M. Bagley. 2011. Selection for Cry3Bb1 resistance in a genetically diverse population of nondiapausing western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 104: 1038-1044.

Oyediran, I. O., B. E. Hibbard, and T. L. Clark. **2004**. Prairie grasses as hosts of the western corn rootworm (Coleoptera: Chrysomelidae). Environ. Entomol. 33: 740–747.

Pardo-López, L., M. Soberón, and A. Bravo. **2013**. *Bacillus thuringiensis* insecticidal three-domain Cry toxins: mode of action, insect resistance and consequences for crop protection. FEMS Microbiol. Rev. 37: 3–22.

Parimi, S., L. J. Meinke, B. Wade French, L. D. Chandler, and B. D. Siegfried. **2006**. Stability and persistence of aldrin and methyl-parathion resistance in western corn rootworm populations (Coleoptera: Chrysomelidae). Crop Protection. 25: 269–274.

Pereira, A. E., D. Souza, S. N. Zukoff, L. J. Meinke, and B. D. Siegfried. **2017**. Cross-resistance and synergism bioassays suggest multiple mechanisms of pyrethroid resistance in western corn rootworm populations. PLoS ONE. 12.

Pereira, A. E., H. Wang, S. N. Zukoff, L. J. Meinke, B. W. French, and B. D. Siegfried. 2015. Evidence of field-evolved resistance to bifenthrin in western corn rootworm (*Diabrotica virgifera virgifera* LeConte) populations in western Nebraska and Kansas. PLoS ONE 10: e0142299.

Petzold-Maxwell, J. L., A. P. Alves, R. E. Estes, M. E. Gray, L. J. Meinke, E. J. Shields, S. D. Thompson, N. A. Tinsley, and A. J. Gassmann. **2013**. Applying an integrated refuge to manage western corn rootworm (Coleoptera: Chrysomelidae): effects on survival, fitness, and selection pressure. J. Econ. Entomol. 106: 2195–2207.

Pierce, C. M., and M. E. Gray. **2006**. Seasonal oviposition of a western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), variant in east central Illinois commercial maize and soybean fields. Environ. Entomol. 35: 676–683.

Pruess, K. P., J. F. Witkowski, E. S. Raun. 1974. Population suppression of western corn rootworm by adult control with ULV malathion. J. Econ. Entomol. 5:651–655.

Quiring, D. T., and P. R. Timmins. **1990**. Influence of reproductive ecology on feasibility of mass trapping *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). J. Appl. Ecol. 27: 965.

Reinders, J. D., B. D. Hitt, W. W. Stroup, B. W. French, and L. J. Meinke. 2018. Spatial variation in western corn rootworm (Coleoptera: Chrysomelidae) susceptibility to Cry3 toxins in Nebraska. PLoS ONE 13: e0208266.

Rondon, S. I., and M. E. Gray. **2004**. Ovarian development and ovipositional preference of the western corn rootworm (Coleoptera: Chrysomelidae) variant in east central Illinois. J. Econ. Entomol. 97: 390–396.

Roselle, R. E., L. W. Anderson, R. G. Simpson, and M. C. Webb. 1959. Annual report for 1959, Cooperative Extension Work in Entomology, Lincoln, Nebraska.

Roselle, R. E., L. W. Anderson, R. C. Simpson, and P. W. Bergman. 1960. Annual report for 1960, Cooperative Extension Work in Entomology, Lincoln, Nebraska.

Roselle, R. E., L. W. Anderson, and P. W. Bergman. 1961. Annual report for 1961, Cooperative Extension Work in Entomology, Lincoln, Nebraska.

Russell, R. M., and J. L. Robertson. 1979. Programming probit analysis. Bull. Entomol. Soc. Am. 25: 191–193.

Schaafsma, A. W., G. H. Whitfield, and C. R. Ellis. 1991. A temperature-dependent model of egg development of the western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae). Can. Entomol. 123(6): 1183-1197.

Schnepf, E., N. Crickmore, J. Van Rie, D. Lereclus, J. Baum, J. Feitelson, D. R. Zeigler, and D. H. Dean. **1998**. *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiol. Mol. Biol. Rev. 62: 775–806.

Schrader, P. M., R. E. Estes, N. A. Tinsley, A. J. Gassmann, and M. E. Gray. 2017. Evaluation of adult emergence and larval root injury for Cry3Bb1-resistant populations of the western corn rootworm. J. Appl. Entomol. 141: 41-52.

Shaw, J. T., J. H. Paullus, and W. H. Luckmann. 1978. Corn rootworm oviposition in soybeans. J. Econ. Entomol. 71: 189-191.

Short, D. E., and R. E. Hill. **1972**. Adult emergence, ovarian development, and oviposition sequence of the western corn root worm in Nebraska. J. Econ. Entomol. 65: 685–689.

Shrestha, R. B., and A. J. Gassmann. 2020. Inheritance and fitness costs of Cry3Bb1 resistance in diapausing field strains of western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 113(6): 2873-2882.

Shrestha, R. B., M. W. Dunbar, B. W. French, and A. J. Gassmann. **2018**. Effects of field history on resistance to Bt maize by western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae). PLoS ONE. 13.

Souza, D., B. C. Vieira, B. K. Fritz, W. C. Hoffmann, J. A. Peterson, G. R. Kruger, and L. J. Meinke. 2019. Western corn rootworm pyrethroid resistance confirmed by aerial application simulations of commercial insecticides. Sci. Rep. 9: 6713.

Souza, D., J. A. Peterson, R. J. Wright, and L. J. Meinke. 2020. Field efficacy of soil insecticides on pyrethroid-resistant western corn rootworms (*Diabrotica virgifera virgifera* LeConte). Pest Manag. Sci. 76: 827-833.

Spencer, J. L., B. E. Hibbard, J. Moeser, and D. W. Onstad. **2009**. Behaviour and ecology of the western corn rootworm (*Diabrotica virgifera virgifera* LeConte). Agricultural and Forest Entomology. 11: 9–27.

Spencer, J. L., E. Levine, S. A. Isard, and T. R. Mabry. **2005**. Movement, dispersal and behavior of western corn rootworm in rotated maize and soybean fields, pp. 121–144. *In* Western Corn Rootworm: Ecology and Management. CABI Pub., Wallingford, Oxfordshire.

Spencer, J. L., S. A. Hughson, and E. Levine. **2014**. Insect resistance to crop rotation, pp. 233–278. *In* Insect Resistance Management: Biology, Economics, and Prediction. Elsevier, Amsterdam.

Spencer, J. L., T. R. Mabry, and T. T. Vaughn. 2003. Use of transgenic plants to measure insect herbivore movement. J. Econ. Entomol. 96: 1738–1749.

Spike, B. P., and J. J. Tollefson. 1991. Response of western corn rootworm-infested corn to nitrogen fertilization and plant density. Crop Sci. 31(3): 776-785.

Storer, N. P. 2003. A spatially explicit model simulating western corn rootworm (Coleoptera: Chrysomelidae) adaptation to insect-resistant maize. J. Econ. Entomol. 96: 1530–1547.

Sutter, G. R., J. R. Fisher, N. C. Elliott, and T. F. Branson. **1990**. Effect of insecticide treatments on root lodging and yields of maize in controlled infestations of western corn rootworms (Coleoptera: Chrysomelidae). J. Econ. Entomol. 83: 2414–2420.

Sutter, G. R., T. F. Branson, J. R. Fisher, and N. C. Elliott. **1991**. Effect of insecticides on survival, development, fecundity, and sex ratio in controlled infestations of western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 84: 1905–1912.

Tabashnik, B. E., and F. Gould. **2012**. Delaying corn rootworm resistance to Bt corn. J. Econ. Entomol. 105: 767–776.

Tabashnik, B. E., M. S. Sisterson, P. C. Ellsworth, T. J. Dennehy, L. Antilla, L. Liesner, M. Whitlow, R. T. Staten, J. A. Fabrick, G. C. Unnithan, A. J. Yelich, C. Ellers-Kirk, V. S. Harpold, X. Li, and Y. Carrière. **2010**. Suppressing resistance to Bt cotton with sterile insect releases. Nat. Biotechnol. 28: 1304–1307.

Tabashnik, B. E., Y. Carrière, T. J. Dennehy, S. Morin, M. S. Sisterson, R. T. Roush, A. M. Shelton, and J.-Z. Zhao. **2003**. Insect resistance to transgenic Bt crops: lessons from the laboratory and field. J. Econ. Entomol. 96: 1031–1038.

Tate, H. D., and O. S. Bare. 1946. Corn rootworms. Nebraska Agric. Exper. Station Bulletin 381: 1-12.

Tinsley, N. A., R. E. Estes, and M. E. Gray. **2014a**. Control of corn rootworm beetles with foliar-applied insecticides, 2013. Arthropod Manag. Tests. 39. doi.org/10.4182/amt.2014.F42.

Tinsley, N. A., R. E. Estes, and M. E. Gray. **2014b**. Control of corn rootworm beetles with foliar-applied insecticides and insecticide/fungicide combinations, 2013. Arthropod Manag. Tests. 39. doi.org/10.4182/amt.2014.F41.

Tollefson, J. J. 1986. Accelerated degradation of soil insecticides used for corn rootworm control, pp. 1131-1136. *In* Proceedings of the British Crop Protection Conference Pests and Diseases, Brighton Metropole, UK.

Urías-López, M. A., and L. J. Meinke. 2001. Influence of western corn rootworm (Coleoptera: Chrysomelidae) larval injury on yield of different types of maize. J. Econ. Entomol. 94: 106-111.

(USEPA) US Environmental Protection Agency. 2011. Biopesticides registration action document. MON 89034 x TC1507 x MON 88017 x DAS-59122-7 (SmartStax®) B.t. corn seed blend. (https://www3.epa.gov/pesticides/chemsearch/regactions/pip/smartstax-seedblend.pdf) (accessed 15 June 2021).

(USEPA) US Environmental Protection Agency. 2012. Pesticide product label, 5307 corn. (https://www3.epa.gov/pesticides/chem_search/ppls/067979-00022-20120731.pdf) (accessed 15 June 2021).

(USEPA) US Environmental Protection Agency. 2013. Pesticide product label, Bt11 x MIR162 x MIR604 x TC1507 x 5307 5% refuge seed blend corn. (https://www3.epa.gov/ pesticides/chemsearch/ppls/067979-00026-20130802.pdf) (accessed 15 June 2021).

van Rozen, K., and A. Ester. 2010. Chemical control of *Diabrotica virgifera virgifera* LeConte. J. Appl. Entomol. 134: 376-384.

Wangila, D. S., A. J. Gassmann, J. L. Petzold-Maxwell, B. W. French, and L. J. Meinke. 2015. Susceptibility of Nebraska western corn rootworm (Coleoptera: Chrysomelidae) populations to Bt corn events. J. Econ. Entomol. 108: 742-751.

Wangila, D. S., B. R. Leonard, M. N. Ghimire, Y. Bai, L. Zhang, Y. Yang, K. D. Emfinger, G. P. Head, F. Yang, Y. Niu, and F. Huang. **2013**. Occurrence and larval movement of *Diatraea saccharalis* (Lepidoptera: Crambidae) in seed mixes of non-Bt and Bt pyramid corn. Pest Manag. Sci. 69: 1163–1172.

Wilde, G. E. 1971. Temperature effect on development of western corn rootworm eggs. J. Kansas Entomol. Soc. 44(2): 185-187.

Wilde, G. E. 1999. Areawide management of corn rootworm in Kansas. *In* Summary of the Abstracts, 4th FAO WCR/TCP meeting, 5th EPPO ad hoc Panel and 6th International IWGO Workshop on *Diabrotica virgifera virgifera*. LeConte, Paris, France, pp. 26–27.

Weiss, M. J., K. P. Seevers, and Z. B. Mayo. 1985. Influence of western corn rootworm larval densities and damage on corn rootworm survival, developmental time, size and sex ratio (Coleoptera: Chrysomelidae). J. Kansas Entomol. Soc. 58: 397-402.

Wright, R. J., L. J. Meinke, and K. J. Jarvi. 1999. Corn rootworm management. *In* Historical Materials from University of Nebraska-Lincoln Extension. 1084. Univ. Nebr. Coop. Ext.

Wright, R. J., M. E. Scharf, L. J. Meinke, X. Zhou, B. D. Siegfried, and L. D. Chandler. 2000. Larval susceptibility of an insecticide-resistant western corn rootworm (Coleoptera: Chrysomelidae) population to soil insecticides: laboratory bioassays, assays of detoxification enzymes, and field performance. J. Econ. Entomol. 93(1): 7-13.

Zhu, K. Y., G. E. Wilde, P. E. Sloderbeck, L. L. Buschman, R. A. Higgins, R. J. Whitworth, R. A. Bowling, S. R. Starkey, and F. He. **2005**. Comparative susceptibility of western corn rootworm (Coleoptera: Chrysomelidae) adults to selected insecticides in Kansas. J. Econ. Entomol. 98: 2181–2187.

Zukoff, S. N., K. R. Ostlie, B. Potter, L. N. Meihls, A. L. Zukoff, L. French, M. R. Ellersieck, B. W. French, and B. E. Hibbard. 2016. Multiple assays indicate varying levels of cross resistance in Cry3Bb1-selected field populations of the western corn rootworm to mCry3A, eCry3.1Ab, and Cry34/35Ab1. J. Econ. Entomol. 109: 1387-1398.

CHAPTER 2: EFFECT OF FOLIAR APPLICATION OF INSECTICIDE ON WESTERN CORN ROOTWORM (COLEOPTERA: CHRYSOMELIDAE) POPULATION DENSITY IN NORTHEAST NEBRASKA

2.1. Introduction

The western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte, is one of the most economically important insect pests of field maize (*Zea mays* L.) in the United States Corn Belt and is an annual management challenge in Nebraska continuous maize (≥ 2 successive years of cultivation). The yield loss from WCR damage and control costs can exceed $2 billion dollars annually (Wechsler and Smith 2018). The WCR is univoltine, with all immature life stages occurring within the soil (Krysan 1986). Larvae only feed on roots of specific grass species but maize is the primary host in agricultural systems (Clark and Hibbard 2004, Meinke et al. 2021). Larval injury to maize roots may decrease the plant's ability to obtain nutrients and water, thus reducing plant growth, stability, and yield (Kahler et al. 1985, Spike and Tollefson 1991, Godfrey et al. 1993, Hou et al. 1997, Urías-López et al. 2000, Urías-López and Meinke 2001 Tinsley et al. 2013). Following the node-injury scale developed by Oleson et al. (2005), each composite node of root injury can result in a yield loss of 15-17% (Dun et al. 2010, Tinsley et al. 2013). Adults feed primarily on maize ear tissues and pollen (Ball 1957, Levine and Oloumi-Sadeghi 1991). When high adult densities are present, silk clipping damage can occur which can reduce pollination and fertilization of maize resulting in loss of kernels and reduced grain yield (Chiang 1973, Culy et al. 1992; Meinke 2014).

WCR integrated pest management programs (IPM) include multiple tactics to reduce WCR population densities and associated crop injury. These tactics include crop rotation to a non-host crop (i.e., maize-soybean rotation), chemical control (foliar and soil insecticides), and transgenic maize hybrids (insecticidal protein expression) (Levine & Oloumi-Sadeghi 1991, Wright et al. 1999, Wangila et al. 2015). Transgenic maize, expressing the rootworm insecticidal toxin(s) derived from *Bacillus thuringiensis* Berliner (Bt), is the most recent WCR control tactic used extensively to manage WCR infesting continuous maize fields. The Bt toxins Cry34/35Ab1, mCry3A, and Cry3Bb1were made commercially available for WCR control in the US during the 2000s and originally marketed as single traits (Devo et al. 2013, Narva et al. 2013, Wangila et al, 2015). Transgenic maize has largely replaced soil insecticides as the primary management tactic in the U.S. Corn Belt due to its increased efficacy and ease of use (Rice 2004, Meinke et al. 2021).

The extensive reliance of growers on Bt traits to manage rootworms has led to WCR evolving resistance to Bt traits (Hellmich 2012, Andow et al. 2016). Rapid evolution of resistance has been documented in lab selection experiments and the field when selection pressure is maintained over multiple generations (Meihls et al. 2008, Oswald et al. 2011, Frank et al. 2013, Gassmann et al. 2014). Bt resistance has evolved after consecutive use of the same Bt trait for only 3 or more years in the field (Gassmann et al. 2011, Wangila et al. 2015) with a positive correlation between number of years Bt-maize is grown with survival of WCR populations in Bt-maize laboratory assays (Gassmann et al. 2011, 2012; Reinders et al. 2018). Resistance to Cry3 traits and Cry34/35Ab1 has been documented in local areas of the US Corn Belt (Gassmann et al. 2011, 2021; Wangila et al. 2015, Ludwick et al. 2016, Schrader et al. 2017, Zukoff et al. 2016, Shrestha et al. 2018). Due to evolved resistance to single-trait Bt toxins, pyramids containing two or more insecticidal traits are replacing single-trait corn hybrids as commercial products (Gassmann et al. 2014, Reinders et al. 2018).

Research and development of new protein traits is expensive; therefore, companies have formed cooperative business agreements to share technology and create pyramids (e.g., Cry3Bb1 + Cry34/35Ab1 (USEPA 2011), mCryA + eCry3.1Ab (USEPA 2012), mCry3A + Cry34/35 Ab1 (USEPA 2013)). More than one mode of action within a single plant can delay WCR evolution of resistance by independently acting on the same insect (Zhao et al 2003, Onstad and Meinke 2010, Storer et al. 2012, Keweshan et al. 2015, Andow et al 2016). For example, SmartStax® (Cry3Bb1 + Cry34/35Ab1), the first transgenic pyramided maize provided greater root protection than single trait hybrids when initially commercialized (Head et al. 2014).

Despite the increased effectiveness of pyramids against WCR, all current pyramids contain one or more traits that were originally sold as single trait products. Previous exposure of WCR populations to single traits and confirmed single trait resistance in some WCR populations can potentially compromise current commercial pyramids (Gassmann et al. 2012, 2014, Calles-Torrez et al. 2019). If resistance to one of the toxins is present within a population, the resistant trait exposes the second trait to increased selection pressure (Gould et al. 2006, Onstad and Meinke 2010). Cross-resistance, such as documented for Cry3Bb1 and mCry3A, also prevents rotation of traits as an effective insect resistant management (IRM) strategy to mitigate resistance evolution (Gassmann et al. 2012, 2014). The Bt trait, Cry34/35Ab1, has been included in pyramided hybrids with Cry3 toxins because it is not cross-resistant with Cry3 traits (Gassmann et al. 2012, 2016, Wangila et al. 2015). Extending the durability and efficacy of current Bt hybrids is paramount to bridge the transition to new control tactics and products that may be commercialized in the next decade. There is a need to integrate transgenic maize with existing IPM tactics to develop effective IRM programs that delay or mitigate resistance.

Therefore, as part of a larger project conducted in northeast Nebraska to assess the value of applying integrated management tactics to mitigate potential changes in WCR susceptibility to rootworm-active Bt traits this study evaluated the effectiveness of foliar-applied insecticides as a potential best management practice to suppress adult WCR densities and potential larval pressure when planting Bt hybrids the following season. The objectives of the study sought to 1) evaluate within season efficacy of foliar-applied insecticides, 2) determine the potential effect of foliar insecticide applications on WCR density the following season, and 3) determine the potential relationship between the most commonly used adult WCR sampling techniques, whole plant counts (WPC) and unbaited sticky traps.

2.2. Materials and Methods

2.2.1. Field Criteria:

All fields were located in northeast Nebraska where confined livestock production and continuous maize were common. Maize fields were selected under the criteria of moderate-to-high WCR population density with a history of planting rootworm-active Bt maize hybrids continuously for a minimum of 3 years which made them at risk for WCR resistance evolution to Bt traits. Many fields had a history of 5-10+ years of continuous maize production. In 2019, foliar insecticides were applied to five commercial fields during the WCR beetle activity period and seven fields were untreated controls to evaluate within-season efficacy of the insecticides used (Fig. 2.1, Table 2.1). In 2020, the study was repeated with four commercial fields selected as insecticide treatment fields and five selected as untreated fields (Fig. 2.1, Table 2.2). Insecticide used for treated fields was either a pyrethroid, organophosphate (OP), or OP/pyrethroid tank mix: choice of foliar application was left to grower discretion (Tables 2.1, 2.2). An OP/pyrethroid tank mix was used in 4 of 5 and 3 of 5 fields in 2019 and 2020, respectively (Tables 2.1, 2.2).

2.2.2. Sampling:

In 2019-2020, whole plant counts (WPCs) were used to estimate the population density of WCR in each field with unbaited Pherocon AM traps used as a complement to WPCs to estimate sex ratios and proportion gravid females present. All fields were sampled using WPCs, with sticky traps included in a subset of fields (Tables 2.1, 2.2). Twelve sites were sampled in a random section of each field with all sites placed >30-40 m apart for counts to be independent samples (Hein and Tollefson 1984, 1985a; Midgarden et al. 1993; Darnell et al. 1999). Sampling was conducted during the R2-R3 maize growth stage during peak beetle emergence in all fields. Sampling of foliar insecticide treated fields was performed to estimate the potential change in density before and after insecticide application; untreated fields were sampled as controls to estimate if significant changes in WCR population density occurred in the absence of insecticide application.

Whole Plant Count Sampling Method: The WPC technique is the sampling method commonly used by agricultural consultants in Nebraska for estimating beetle density when making rootworm management decisions (Darnell et al. 1999). WPC is a visual sampling method that consists of counting all WCR adults on the maize plant, from the bottom-to-top of the plant and within each ear. WPC data were collected midmorning to early afternoon within the temperature range: 23-28^{o}C. Each WPC was performed by the same individual for consistent data collection and to reduce sampling variation. Three random WPCs were made at each of the 12 sites per field to obtain a composite WPC per site (36 total WPC per field). The same procedures were followed in 2019 and 2020.

In 2019, WPCs were obtained in 12 fields, twice per field in 7 counties in Northeast Nebraska (Fig. 2.1). In 2020, 9 fields were sampled in 6 counties (Fig. 2.1). Variation in fields included in the study between years was dictated by previously described field criteria (e.g., some fields were rotated to soybean in 2020) and farmer intent to apply foliar insecticide.

Pherocon® AM Unbaited Sticky Traps: Yellow Pherocon AM traps have been used as an alternative to WPCs to estimate WCR densities and develop economic threshold indices (Hein and Tollefson 1984, 1985a; Karr and Tollefson 1987; O'Neal et al. 2001; Pierce and Gray 2007). When deployed in fields during this study, a single sticky trap was placed at each of the 12 sites per field on the same day WPCs were taken. The sticky traps were 27.9 x 22.4 cm in size and were folded around the ear-zone. Sticky traps were left within fields for 5–7-day intervals, except field FD10 which was left for 3 days, before being collected. In 2019, traps were placed in most fields for at least one sampling interval; five fields were sampled during two intervals and six fields one interval (Table 2.1). In 2020, seven fields were sampled for two intervals (Table 2.2). Data was collected from most fields prior to insecticide application and a subset of fields after insecticide application to provide a dataset that covered a broad range of WCR densities that could be compared to WPCs that were obtained.

The number of WCR adults on each trap was documented along with the sex ratio of WCR in each trap. Males were identified by the presence of adhesive hairs on the mesothoracic tarsomere used to grasp females during mating (Hammack and French 2007). A second characteristic used was the extra sclerite at the abdominal apex of males (White 1977). Females were identified by absence of male morphological traits. Additionally, a yellow, swollen abdomen or visible eggs were used to confirm gravid females.

Emergence Cage Sampling Method: In 2020, single-plant emergence cages were placed in a subset of fields sampled during 2019 that were planted back to maize to document survival to the adult stage (Table 2.3). Emergence cages were a modification of the design described by Fisher et al. (1980) and Hein et al. (1985b) but allowed the caged plant to remain intact and grow up through the center of the cage (Pierce and Gray 2007). Adult emergence was captured in a glass jar placed at the top of each cage. A 4-row (3.1 m) x 61.5 m strip planted to a VT Double PRO® (VT2P) hybrid was placed in a subset of each field that was sampled in 2019. The hybrid did not express any rootworm-active Bt traits. Four cages spaced 10-15 m apart were placed in each strip and emerged beetles were collected weekly throughout the adult emergence period. Data obtained from emergence cages was used to determine if foliar insecticide application the previous year decreased the WCR population density the following year in maize fields.

2.3. Statistical Analysis:

Whole Plant Count Analysis: For WPC statistical analysis, a field site mean was calculated by taking the average of 3 WPCs per site. The average of 12 site means per field represented the mean WPC of a field. The mean WPCs were then analyzed as counts for proportion change in mean WCR before and after insecticide application. The proportional change inherently accounts for any effect of insecticide application through the scaled difference in mean WPC. Since there were some untreated fields that had a higher mean WPC at time point 02 versus 01, the variable could not be treated as a simple proportion. Therefore, proportion change was modeled using PROC GLIMMIX in a Gaussian distribution. Normality was confirmed based on the proportion residual plots. A blocked design was used with insecticide as treatment and year as a blocking factor. The Type III Fixed Effects (PROC GLIMMIX) was performed on the fixed effects of year, treatment, and year x treatment interaction.

The mean WCR whole plant counts from 2019 fields that were replanted to maize during 2020 (5 treated, 3 untreated; Table 2.3) and 2020 sampled fields (Table 2.2) were analyzed to compare estimated adult densities before and after insecticide application. The experimental design followed a split-plot in time, whole plot unit represented by field, and spilt plot experimental unit being the before/after state of each field after blocking by year. The generalized chi-square/df test was used to check whether there was overdispersion in the chosen model. The fixed effect of year (2019 and 2020), time (before and after), insecticide (presence or absence), and their interactions were analyzed using a Type III Tests of Fixed Effects (PROC GLIMMIX). All data were analyzed using SAS® software version 9.4 (SAS/STAT version 15.1).

Female: Male Ratio Analysis: The pre- insecticide and time 01 female: male counts per field from sticky traps in 2019 and 2020 were analyzed to determine if sex ratios were similar in treated and untreated fields prior to insecticide application. Sex ratio data was averaged and divided by the amount of time traps were left in a field for a mean female: male count per day. The mean female: male count per day represented the average WCR sex ratio per field. The experimental design was a blocked design with insecticide as treatment and year as blocking factor. The Type III Fixed Effects (PROC GLIMMIX) was performed on the fixed effects of year, treatment, and year x treatment interaction and was normally distributed. Sex ratio was analyzed using SAS® software version 9.4 (SAS/STAT version 15.1).

Proportion Gravid Analysis: The proportion gravid females from sticky traps for pre-application and time 01 periods was analyzed to determine if proportion gravid was similar in treated and untreated fields prior to insecticide application. Proportion of gravid WCR was obtained by taking the mean total gravid female divided by mean total female counts of a field. The experimental design was the same as described for the sex ratio analysis except the analysis was conducted as a binomial distribution.

Sticky Trap vs. WPC Relationship: To determine if there was a consistent relationship between mean WCR WPC and mean WCR per trap per day, data from 2019 and 2020 was compiled together to conduct correlation and regression analyses. Each sample observation consisted of a mean WPC and the mean daily trap count per field from the interval starting the day the WPC was recorded (n = 24). Individual sample observations included intervals before and after foliar application, and time 01, and time 02 intervals in control fields. The variability between fields was accounted for by a field random effect since there were some fields that had more than one sampling interval. The Pearson correlation coefficient between mean whole plant count and daily mean trap count was calculated using PROC CORR in SAS Software version 9.4. A regression of mean daily trap counts on mean WPCs was implemented in PROC GLIMMIX using SAS v. 9.4 (SAS/STAT version 15.1).

Emergence Cage Analysis: An analysis was conducted on the total season adult WCR emergence from four cages in each field. The analysis was implemented using PROC GLIMMIX in SAS v. 9.4 (SAS/STAT version 15.1). and run as a generalized linear model following a negative binomial distribution with a log link function due to the count nature of the response. The model compared adult emergence from categories insecticide treated versus untreated control fields.

2.4. Results

2.4.1. Whole-Plant Count Analysis

Proportion Change: Proportional change in mean WPC was significantly affected by treatment (Table 2.4). The year and treatment x year interaction were not significant indicating that results followed a similar trend each year (Table 2.4). Proportional change in mean WPC of treatment fields was significantly different from untreated fields (2019: treated: -0.92 ±0.05, untreated: -0.01 ±0.04; 2020: treated: -0.90

±0.06, untreated: 0.01 ±0.05). Proportion change in treated fields ranged from -0.7 to -1.00 (2019) and -0.78 to -1.00 (2020) in treated fields and -0.19 to +0.15 (2019) and -0.08 to +0.13 (2020) in untreated control fields (Table 2.5).

Mean Whole Plant Count: The mean WPC was significantly affected by year, time, treatment, and the time x treatment interaction (Table 2.6). Time x year, treatment x year, and time x treatment x year interactions were not significant indicating that a similar trend was observed each year. The significant reduction in mean whole plant count in treated fields after insecticide application but not in the untreated control led to the time by treatment interaction (Fig. 2.2). This result was very consistent across fields in each treatment (Fig. 2.3, 2.4).

2.4.2. Pherocon® Sticky Trap Analyses

Female: Male Sex Ratio: Sex ratios of WCR in pre- insecticide application and untreated time 01 treatments were significantly affected by year but not insecticide or the year x insecticide interaction (Table 2.7). Results indicate that the overall mean sex ratio was different between years, but treated and untreated fields were similar in sex ratio prior to insecticide treatment. Sex ratios prior to insecticide application were more skewed toward females in 2019 than that recorded in 2020 (overall mean female /male ratios for treated pre + untreated 01: 2019: 1.35; 2020: 0.72).

Proportion Gravid: Results of the analysis of proportion gravid of pre- and time 01 treatment fields paralleled results obtained from the sex ratio analysis. Proportion gravid was significantly affected by year but not insecticide or the year x insecticide interaction (Table 2.8). Results indicate proportion gravid was greater in 2019 than 2020 but was not significantly different between treatments prior to insecticide application in either year (Table 2.3, 2.9).

2.4.3. Whole Plant Counts: Sticky Trap Relationship

Correlation analysis revealed a significant positive association (p=0.0071) between mean WPC and sticky trap capture per day. The estimated Pearson correlation coefficient was 0.48915. The linear regression analysis also produced a significant positive relationship (p=0.0390) between both sampling methods. The resulting equation for the line of best fit was:

$$\widehat{Trap} = 2.4335 + 2.2011 * WPC$$

with 2.4335 as the intercept and 2.2011 the slope (Fig. 2.5).

2.4.4. Emergence Cage Comparison

Comparison of total adult emergence from 2019 insecticide treated and untreated fields planted back to maize in 2020 showed a significant difference between treated vs. untreated fields *(P*=0.0223); mean total emergence was greater in untreated than treated fields. Untreated fields produced an average total season emergence of 235 ± 51.1 adults per field from four emergence cages while treated fields produced an average total season emergence of 90 ± 20.1 per field.

2.5. Discussion

The significant proportional change in mean WCR WPCs and associated reduction of WCR plant counts after foliar application of pyrethroid and/or organophosphate insecticides clearly demonstrates that foliar application of insecticides targeting adult WCR effectively reduced the within season adult population density in northeast Nebraska maize fields. Foliar insecticides in the study were effectively applied with three different techniques (airplane, ground rig, and chemigation), providing 70-100% reduction in WCR density.

In previous diagnostic bioassays (LC_{99}) conducted with bifenthrin in northeast Nebraska, 59-88%, and 70% mortality, respectively, was reported from WCR populations in Cuming, and Pierce Counties confirming some resistance alleles were present (Pereira et al. 2015). Despite this, the WPC field data from this study documented effective control of WCR in Cuming Co. with formulated bifenthrin (F2: 95% mortality) and as a tank mix with an OP (F5: 100% mortality) (Table 2.5). The level of control with formulated bifenthrin in this study is comparable to results reported from field trials conducted in south central Nebraska (93-100% WCR density reduction) (Seymour et al. 1997, DeVries et al. 2016, Tinsley et al. 2016). In contrast, reduced susceptibility of WCR to bifenthrin in both active ingredient and formulated product bioassays has been documented from populations in southwestern Nebraska and several areas of Kansas (Zhu et al. 2005, Pereira et al. 2015, Souza et al. 2019). Collectively, these results indicate that WCR populations in the eastern half of Nebraska are still relatively susceptible to bifenthrin.

Other than the extensive literature published on adult WCR field-evolved resistance to the OP methyl parathion that occurred in south-central Nebraska during the 1990s (Meinke et al. 1998, Meinke et al. 2021) little information is available on current adult WCR susceptibility to OP insecticides. This study partially fills this void with data on chlorpyrifos which was the product of choice by many grower cooperators either as a stand-alone product or in a tank mix with a pyrethroid. With the exception of FD6, chlorpyrifos tank-mixed with either bifenthrin or lambda-cyhalothrin reduced WCR densities post-application by 94-100%. This is similar to that reported for chlorpyrifos/lambda-cyhalothrin tank mix by Tinsley et al. 2014a, 2014b (95-100% control). Singular chlorpyrifos treatment in FD6 and FD10 provided 78-88% control which was lower than reported in Illinois trials (100% control, Tinsley et al. 2014b). Overall, with the possible exception of the Boone County populations, WCR in northeast Nebraska appear to be susceptible to chlorpyrifos.

In Boone County, field 6 treated with chlorpyrifos/lambda-cyhalothrin tank-mix in 2019 and formulated chlorpyrifos in 2020 exhibited the lowest level of control (74%, 78% respectively). The chlorpyrifos/lambda-cyhalothrin tank-mix provided excellent control in FD10 during 2019 (98% reduction) but lower efficacy was obtained in 2020 when treated with only chlorpyrifos (88% control, Tables 2.1. 2.2. 2.5). The reduction in efficacy compared to other populations in this study and other published data (Tinsley et al. 2014a, 2014b) suggests that the sampled populations from Boone Co. have evolved a low level of resistance to bifenthrin and chlorpyrifos (FD6) or chlorpyrifos (FD10). This hypothesis is supported by baseline susceptibility lab bioassays of populations from fields FD6 and FD10 with bifenthrin and chlorpyrifos (chapter 3).

The contrast in sex ratios and proportion gravid females between years obtained from sticky traps prior to insecticide application suggests that WCR phenology was more advanced in 2019 than 2020 despite sampling during a similar maize phenology period (R2-R3) (Tables 2.3, 2.9). In general, sampling was conducted at least one-two weeks later in 2019 than in 2020 (Tables 2.1, 2.2) which shifted most sampling into August.

Because mean male emergence occurs earlier than mean female emergence (protandry, Branson 1987) sex ratios typically are skewed more toward males early in the WCR activity period and shift more toward females later in the season (Short and Hill 1972, Godfrey and Turpin 1983, Darnell et al. 2000). The general bias toward male capture on sticky traps recorded in 2020 is typical of what has been reported especially when trap catches are compared to aspirated collections over time during the season (Godfrey and Turpin 1983, Kuhar and Youngman 1995). However, Godfrey and Turpin (1983) did report a shift from male bias to female bias on sticky traps during mid-late August sampling periods. The later average sampling period during this study in 2019 appears to have followed the same pattern.

Although the WPC sampling technique is the most used method by ag-consultants (Godfrey and Turpin 1983, Kuhar and Youngman 1995, Darnell et al. 1999) renewed interest in using Pherocon AM sticky traps to monitor WCR has emerged. Agricultural companies have developed incentive programs to encourage growers to monitor their fields via sticky traps (e.g., Bayer 2021). The WPC and sticky trap densities recorded tracked well in this study, as indicated by the positive relationship between the two techniques that was derived (Fig. 2.5). More research would be needed to verify this positive relationship in more environments, but the modeled equation based on the range of WPCs sampled in the Nebraska environment could provide estimates of either WPC or trap numbers from the other.

Mean WCR population densities, sex ratios, and proportion gravid were all similar in 2019 treated and untreated fields prior to insecticide application which allowed a direct comparison of adult survival in treated and untreated fields planted back to maize in 2020. WCR densities obtained in emergence cages from the VT2P hybrid expressed no rootworm Bt-traits, so WCR were only affected by 2019 applications and environmental factors. The significant reduction in mean adult production from 2019 insecticide-treated fields occurred despite less-than-optimal treatment timing in 2019. The high proportion of gravid females present prior to insecticide treatment probably led to a significant level of oviposition before treatment. When applying foliar insecticide with the goal of reducing the WCR population the next season, recommended optimal timing would be during peak emergence with higher female: male sex ratio and a lower proportion gravid to reduce egg laying in the field (Meinke 1995, Meinke 2014). Prescence of 10-15% gravid females in the population is a treatment trigger commonly followed (Wright et al. 1999, Meinke 2014). Application timing in 2020 more closely met this criterion. Though only one year of data was available, results showed a solid trend and suggests that one application during the peak WCR emergence period can significantly reduce the WCR population density the following year.

In conclusion, northeast Nebraska WCR populations were generally susceptible to organophosphates and pyrethroids and the results of this study can serve as a baseline for efficacy of formulated compounds in future studies. Most studies targeting adult WCR only provide within-season efficacy data (e.g., Seymour et al. 1997, DeVries et al. 2016, Tinsley et al. 2014a, 2014b) so the follow-up data on the longer-term impact of foliar insecticides on WCR population density provides evidence that properly used adult foliar insecticides may be a viable best management practice in Bt trait IRM programs. Foliar insecticides may reduce the build-up of WCR populations overtime in continuous maize, thereby, reducing selection pressure on Bt-traits in areas of Bt resistance. One thing to note is that the WCR is very adaptable to selection with broadcast application of foliar insecticides and has a long history of documented field-evolved resistance to insecticides (Meinke et al. 2021). Therefore, this will continue to pose a challenge to IPM as the risk of evolving resistance to both transgenic traits and insecticides remains. The use of foliar insecticides must not be overused to prevent the evolution of resistance to current insecticides. A more holistic approach using multiple tactics within a IPM framework is needed to mitigate the evolution of resistance.

Literature Cited

Andow, D. A., S. G. Pueppke, A. W. Schaafsma, A. J. Gassmann, T. W. Sappington, L. J. Meinke, P. D. Mitchell, T. M. Hurley, R. L. Hellmich, and R. P. Porter. **2016**. Early detection and mitigation of resistance to Bt maize by western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 109: 1–12.

Ball, H. J. 1957. On the biology and egg-laying habits of the western corn rootworm. J. Econ. Entomol. 50: 126–128.

Ball, H. J., and G. T. Weekman. 1963. Differential resistance of corn rootworms to insecticides in Nebraska and adjoining states. J. Econ. Entomol. 56: 553-555.

Bayer. 2021. Watch for the threat of corn rootworm. Innovating extraordinary advances in corn, soybeans, cotton, and specialty crops. (https://traits.bayer.com/Pages/the-watch.aspx) (accessed 2 July 2021).

Branson, T. F. 1987. The contribution of prehatch and posthatch development to protandry in the chrysomelid, *Diabrotica virgifera virgifera*. Entomol. Exp. Appl. 43(3): 205-208.

Calles-Torrez, V., J. J. Knodel, M. A. Boetel, B. W. French, B. W. Fuller, and J. K. Ransom. **2019**. Field-evolved resistance of northern and western corn rootworm (Coleoptera: Chrysomelidae) populations to corn hybrids expressing single and pyramided Cry3Bb1 and Cry34/35Ab1 Bt proteins in North Dakota. J. Econ. Entomol. 112: 1875–1886.

Chiang, H. C. 1973. Bionomics of the northern and western corn rootworms. Annu. Rev. Entomol. 18: 47–72.

Clark, T. L., and B. E. Hibbard. **2004**. Comparison of nonmaize hosts to support western corn rootworm (Coleoptera: Chrysomelidae) larval biology. Environ. Entomol. 33: 681–689.

Culy, M. D., C. R. Edwards, and J. R. Cornelius. **1992**. Minimum silk length for optimum pollination in seed corn production fields. J. Prod. Ag. 5: 387–392.

Darnell, S. J., L. J. Meinke, and L. J. Young. 2000. Influence of corn phenology on adult western corn rootworm (Coleoptera: Chrysomelidae) distribution. Environ. Entomol. 29: 587-595.

Darnell, S. J., L. J. Meinke, L. J. Young, and C. A. Gotway. **1999**. Geostatistical investigation of the small-scale spatial variation of western corn rootworm (Coleoptera: Chrysomelidae) adults. Environ. Entomol. 28: 266–274.

Devos, Y., L. N. Meihls, J. Kiss, and B. E. Hibbard. **2013**. Resistance evolution to the first generation of genetically modified Diabrotica-active Bt-maize events by western corn rootworm: management and monitoring considerations. Transgenic Res. 22: 269–299.

DeVries, T. A., and R. J. Wright. **2016**. Evaluation of foliar applied pyrethroid insecticides for control of adult corn rootworm in corn, 2015. Arthropod Manag. Tests. 41. doi.org/10.1093/amt/tsw095.

Dun, Z., P. D. Mitchell, and M. Agosti. 2010. Estimating *Diabrotica virgifera virgifera* damage functions with field trial data: applying an unbalanced nested error component model. J. Appl. Entomol. 134: 409-419.

Frank, D. L., A. Zukoff, J. Barry, M. L. Higdon, and B. E. Hibbard. **2013**. Development of resistance to eCry3.1Ab-expressing transgenic maize in a laboratory-selected population of western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 106: 2506–2513.

Fisher J. R., 1980. A modified emergence trap for quantitative adult corn rootworm studies (Coleoptera: Chrysomelidae). *J. Kans. Entomol. Soc. 53: 363-366*

Gassmann, A. J. 2016. Resistance to Bt maize by western corn rootworm: insights from the laboratory and the field. Curr. Opin. Insect Sci. 15: 111-115.

Gassmann, A. J. 2021. Resistance to Bt maize by western corn rootworm: effects of pest biology, the pest–crop interaction and the agricultural landscape on resistance. Insects. 12: 136.

Gassmann, A. J., J. L. Petzold-Maxwell, E. H. Clifton, M. W. Dunbar, A. M. Hoffmann, D. A. Ingber, and R. S. Keweshan. 2014. Field-evolved resistance by western corn rootworm to multiple *Bacillus thuringiensis* toxins in transgenic maize. Proc. Nat. Acad. Sci. USA 111: 5141-5146.

Gassmann, A. J., J. L. Petzold-Maxwell, R. S. Keweshan, and M. W. Dunbar. 2011. Field-evolved resistance to Bt maize by western corn rootworm. PLoS ONE 6: e22629.

Gassmann, A. J., J. L. Petzold-Maxwell, R. S. Keweshan, and M. W. Dunbar. 2012. Western corn rootworm and Bt maize: challenges of pest resistance in the field. GM Crops and Food 3: 235-244.

Godfrey, L. D., and F. T. Turpin. 1983. Comparison of western corn rootworm (Coleoptera: Chrysomelidae) adult populations and economic thresholds in first-year and continuous corn fields. J. Econ. Entomol. 76: 1028-1032.

Gould, F., M. B. Cohen, J. S. Bentur, G. G. Kennedy, and J. VanDuyn. **2006**. Impact of small fitness costs on pest adaptation to crop varieties with multiple toxins: a heuristic model. J. Econ. Entomol. 99: 2091–2099.

Hammack, L., and B. W. French. **2007**. Sexual dimorphism of Basitarsi in pest species of *Diabrotica* and *Cerotoma* (Coleoptera: Chrysomelidae). Ann. Entomol. Soc. Am. 100: 59–63.

Head, G., M. Carroll, T. Clark, T. Galvan, R. M. Huckaba, P. Price, L. Samuel, and N. P. Storer. 2014. Efficacy of SmartStax® insect-protected corn hybrids against corn rootworm: the value of pyramiding the Cry3Bb1 and Cry34/35Ab1 proteins. Crop Prot. 57: 38-47.

Hein, G. L., and J. J. Tollefson. **1984**. Comparison of adult corn rootworm (Coleoptera: Chrysomelidae) trapping techniques as population estimators. Environ. Entomol. 13: 266–271.

Hein, G. L., and J. J. Tollefson. **1985a**. Use of the Pherocon AM Trap as a scouting tool for predicting damage by corn rootworm (Coleoptera: Chrysomelidae) larvae. J. Econ. Entomol. 78: 200–203.

Hein, G. L., M. K. Bergman, R. G. Brass, and J. J. Tollefson, 1985b. Absolute sampling technique for corn rootworm (Coleoptera: Chrysomelidae) adult emergence that adjusts to fit common-row spacing. J. Econ. Entomol. 78, 1503-1506.

Hellmich, R. L., and K. A. Hellmich. 2012. Use and impact of Bt maize. Nat. Educ. Knowl. 3: 4.

Hou, X., L. J. Meinke, and T. J. Arkebauer. 1997. Soil moisture and larval western corn rootworm injury: influence on gas exchange parameters in corn. Agron. J. 89: 709-717.

Kahler, A. L., A. E. Olness, G. R. Sutter, C. D. Dybing, and O. J. Devine. 1985. Root damage by western corn rootworm and nutrient content in maize. Agron. J. 77: 769-774.

Karr, L. L., and J. J. Tollefson. **1987**. Durability of the Pherocon AM Trap for adult western and northern corn rootworm (Coleoptera: Chrysomelidae) sampling. J. Econ. Entomol. 80: 891–896.

Keweshan, R. S., G. P. Head, and A. J. Gassmann. **2015**. Effects of pyramided Bt corn and blended refuges on western corn rootworm and northern corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 108: 720–729.

Krysan, J. L. 1986. Introduction: biology, distribution, and identification of pest *Diabrotica*, pp. 1–23. *In* J. L. Krysan, and T. A. Miller (eds.), Methods for the study of pest *Diabrotica*. Springer-Verlag, New York, NY.

Kuhar, T. P., and R. R. Youngman. **1995**. Sex ratio and sexual dimorphism in western corn rootworm (Coleoptera: Chrysomelidae) adults on yellow sticky traps in corn. Environ. Entomol. 24: 1408–1413.

Levine, E., and H. Oloumi-Sadeghi. **1991**. Management of Diabroticite rootworms in corn. Annu. Rev. Entomol. 36: 229–255.

Ludwick, D. C., L. N. Meihls, K. R. Ostlie, B. D. Potter, L. French, and B. E. Hibbard. 2017. Minnesota field population of western corn rootworm (Coleoptera: Chrysomelidae) shows incomplete resistance to Cry34Ab1/Cry35Ab1 and Cry3Bb1. J. Appl. Entomol. 141: 28-40.

Meihls, L. N., M. L. Higdon, B. D. Siegfried, N. J. Miller, T. W. Sappington, M. R. Ellersieck, T. A. Spencer, and B. E. Hibbard. 2008. Increased survival of western corn rootworm on transgenic corn within three generations of on-plant greenhouse selection. Proc. Natl. Acad. Sci. USA 105: 19177-19182.

Meinke, L. J. 1995. Adult corn rootworm management. MP63-C, Agricultural Research Division, Institute of Agriculture and Natural Resources. University of Nebraska, Lincoln, Nebraska.

Meinke, L. J. 2014. Adult corn rootworm suppression: corn rootworm management in the transgenic era. Plant Health Prog. doi.org/10.1094/GROW-COR-12-14-068. http://www.plantmanagementnetwork.org/edcenter/seminars/corn/AdultRootworm/ (accessed on 2 July 2021).

Meinke, L. J., B. D. Siegfried, R. J. Wright, and L. D. Chandler. 1998. Adult susceptibility of Nebraska western corn rootworm (Coleoptera: Chrysomelidae) populations to selected insecticides. J. Econ. Entomol. 91: 594-600.

Meinke, L. J., D. Souza, and B. D. Siegfried. 2021. The use of insecticides to manage the western corn rootworm, *Diabrotica virgifera virgifera*, LeConte: history, field-evolved resistance, and associated mechanisms. Insects. 12:112. DOI: 10.3390/insects12020112.

Meinke, L. J., T. W. Sappington, D. W. Onstad, T. Guillemaud, N. J. Miller, J. Komáromi, N. Levay, L. Furlan, J. Kiss, and F. Toth. 2009. Western corn rootworm (*Diabrotica virgifera virgifera* LeConte) population dynamics. Agr. Forest Entomol. 11: 29-46.

Midgarden, D. G., R. R. Youngman, and S. J. Fleischer. **1993**. Spatial analysis of counts of western com rootworm (Coleoptera: Chrysomelidae) adults on yellow sticky traps in corn: geostatistics and dispersion indices. Environ. Entomol. 22: 1124–1133.

Narva, K. E., B. D. Siegfried, and N. P. Storer. **2013**. Transgenic approaches to western corn rootworm control. Yellow Biotechnol. II. 135–162.

Oleson, J. D., Y.-L. Park, T. M. Nowatzki, and J. J. Tollefson. **2005**. Node-injury scale to evaluate root injury by corn rootworms (Coleoptera: Chrysomelidae). J. Econ. Entomol. 98: 1–8.

O'Neal, M. E., M. E. Gray, S. Ratchliffe, and K. L. Steffey. **2001**. Predicting western corn rootworm (Coleoptera: Chrysomelidae) larval injury to rotated corn with Pherocon AM traps in soybeans. J. Econ. Entomol. 94: 98–105.

Onstad, D. W., and L. J. Meinke. **2010**. Modeling evolution of *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) to transgenic corn with two insecticidal traits. J. Econ. Entomol. 103: 849–860.

Oswald, K. J., B. W. French, C. Nielson, and M. Bagley. 2011. Selection for Cry3Bb1 resistance in a genetically diverse population of nondiapausing western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 104: 1038-1044.

Pereira, A.E., H. Wang, S.N. Zukoff, L. J. Meinke, B. W. French, B. D. Siegfried. 2015. Evidence of field-evolved resistance to bifenthrin in western corn rootworm (*Diabrotica virgifera virgifera* LeConte) populations in western Nebraska and Kansas. PLoS ONE 10(11): e0142299. doi:10.1371/journal.pone.0142299.

Pierce, C. M., and M. E. Gray. **2007**. Population dynamics of a western corn rootworm (Coleoptera: Chrysomelidae) variant in east central Illinois commercial maize and soybean fields. J. Econ. Entomol. 100: 1104–1115.

Reinders, J. D., B. D. Hitt, W. W. Stroup, B. W. French, and L. J. Meinke. 2018. Spatial variation in western corn rootworm (Coleoptera: Chrysomelidae) susceptibility to Cry3 toxins in Nebraska. PLoS ONE 13: e0208266.

Rice, M. E. 2004. Transgenic rootworm corn: assessing potential agronomic, economic, and environmental benefits. Plant Health Prog. doi: 10.1094/PHP-2004-0301-01-RV. (http://www.plantmanagementnetwork.org/sub/php/review/2004/rootworm/)

SAS Institute. 2013. SAS/STAT user’s guide. SAS Institute, Cary, NC.

Schrader, P. M., R. E. Estes, N. A. Tinsley, A. J. Gassmann, and M. E. Gray. 2017. Evaluation of adult emergence and larval root injury for Cry3Bb1-resistant populations of the western corn rootworm. J. Appl. Entomol. 141: 41-52.

Seymour, R. C., J. B. Campbell, and R. J. Wright. **1997**. Control of western corn rootworm and residual pest control, 1997. Arthropod Manag. Tests. 24. doi.org/10.1093/amt/24.1.F34.

Short, D. E., and R. E. Hill. 1972. Adult emergence, ovarian development, and oviposition sequence of the western corn rootworm in Nebraska. J. Econ. Entomol. 65(3): 685-689.

Shrestha, R. B., M. W. Dunbar, B. W. French, and A. J. Gassmann. 2018. Effects of field history on resistance to Bt maize by western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae). PLoS ONE 13: e0200156.

Souza, D., B. C. Vieira, B. K. Fritz, W.C. Hoffmann, J. A. Peterson, G. R. Kruger, L. J. Meinke. 2019. Western corn rootworm pyrethroid resistance confirmed by aerial application simulations of commercial insecticides. Sci. Rpts. 9:6713. doi.org/10.1038/s41598-019-43202-w.

Spencer, J. L., B. E. Hibbard, J. Moeser, and D. W. Onstad. 2009. Behavior and ecology of the western corn rootworm (*Diabrotica virgifera virgifera* LeConte). Agr. Forest Entomol. 11: 9-27.

Spike, B. P., and J. J. Tollefson. 1991. Response of western corn rootworm-infested corn to nitrogen fertilization and plant density. Crop Sci. 31: 776-785.

Storer, N. P., G. D. Thompson, and G. P. Head. **2012**. Application of pyramided traits against Lepidoptera in insect resistance management forBtcrops. GM Crops and Food. 3: 154–162.

Tinsley, N. A., R. E. Estes, and M. E. Gray. 2013. Validation of a nested error component model to estimate damage caused by corn rootworm larvae. J. Appl. Entomol. 137: 161-169.

Tinsley, N. A., R. E. Estes, and M. E. Gray. **2014a**. Control of corn rootworm beetles with foliar-applied insecticides, 2013. Arthropod Manag. Tests. 39. doi.org/10.4182/amt.2014.F42.

Tinsley, N. A., R. E. Estes, and M. E. Gray. **2014b**. Control of corn rootworm beetles with foliar-applied insecticides and insecticide/fungicide combinations, 2013. Arthropod Manag. Tests. 39. doi.org/10.4182/amt.2014.F41.

Urías-López, M. A., and L. J. Meinke. 2001. Influence of western corn rootworm (Coleoptera: Chrysomelidae) larval injury on yield of different types of maize. J. Econ. Entomol. 94: 106-111.

Urías-López, M. A., L. J. Meinke, L. G. Higley, and F. J. Haile. 2000. Influence of western corn rootworm (Coleoptera: Chrysomelidae) larval injury on photosynthetic rate and vegetative growth of different types of maize. Environ. Entomol. 29: 861-867.

(USEPA) US Environmental Protection Agency. 2011. Biopesticides registration action document. MON 89034 x TC1507 x MON 88017 x DAS-59122-7 (SmartStax®) B.t. corn seed blend. (https://www3.epa.gov/pesticides/chemsearch/regactions/pip/smartstax-seedblend.pdf) (accessed 15 June 2021).

(USEPA) US Environmental Protection Agency. 2012. Pesticide product label, 5307 corn. (https://www3.epa.gov/pesticides/chem_search/ppls/067979-00022-20120731.pdf) (accessed 15 June 2021).

(USEPA) US Environmental Protection Agency. 2013. Pesticide product label, Bt11 x MIR162 x MIR604 x TC1507 x 5307 5% refuge seed blend corn. (https://www3.epa.gov/ pesticides/chemsearch/ppls/067979-00026-20130802.pdf) (accessed 15 June 2021).

Wangila, D. S., A. J. Gassmann, J. L. Petzold-Maxwell, B. W. French, and L. J. Meinke. 2015. Susceptibility of Nebraska western corn rootworm (Coleoptera: Chrysomelidae) populations to Bt corn events. J. Econ. Entomol. 108: 742-751.

Wechsler, S., and D. Smith. **2018**. Has Resistance Taken root in U.S. corn fields? demand for insect control. American J. Agric. Econ. 100: 1136–1150.

White, R. 1977. Sexual characters of species of *Diabrotica* (Chrysomelidae: Coleoptera). Ann. Entomol. Soc. Am. 70: 168–168.

Wright, R. J., L. J. Meinke, and K. J. Jarvi. 1999. Corn rootworm management. Univ. of Nebraska Coop. Ext. EC99-1563. (https://digitalcommons.unl.edu/cgi/view content.cgi?article=2082&context= extensionhist) (accessed 20 June 2021).

Zhao, J.-Z., J. Cao, Y. Li, H. L. Collins, R. T. Roush, E. D. Earle, and A. M. Shelton. **2003**. Transgenic plants expressing two *Bacillus thuringiensis* toxins delay insect resistance evolution. Nat. Biotechnol. 21: 1493–1497.

Zhu, K. Y., G. E. Wilde, P. E. Sloderbeck, L. L. Buschman, R. A. Higgins, R. J. Whitworth, R. A. Bowling, S. R. Starkey, and F. He. **2005**. Comparative susceptibility of western corn rootworm (Coleoptera: Chrysomelidae) adults to selected insecticides in Kansas. J. Econ. Entomol. 98: 2181–2187.

Zukoff, S. N., K. R. Ostlie, B. Potter, L. N. Meihls, A. L. Zukoff, L. French, M. R. Ellersieck, B. W. French, and B. E. Hibbard. 2016. Multiple assays indicate varying levels of cross resistance in Cry3Bb1-selected field populations of the western corn rootworm to mCry3A, eCry3.1Ab, and Cry34/35Ab1. J. Econ. Entomol. 109: 1387-1398.

Table 2.1. Background: sampling and insecticide application information per field. 2019.

Field	County	Treatment	Sampling Dates[a]	WPC[b]	Sticky Trap[b]	Field Size (Ha)	Insecticide(s) Applied[c]
FD1	Cuming	Treated	31-July, 5-Aug	Pre/Post	Pre/Post	34.3	2 Aug: 365ml/ha generic bifenthrin (Plane)
FD2	Colfax	Untreated	31-July, 5-Aug	01 02	01 02	56.7	NA
FD3	Pierce	Treated	8-Aug, 13-Aug	Pre/Post	Pre/Post	32.4	10 Aug: 1096ml/ha Colbalt®[1] (Plane)
FD4	Stanton	Untreated	8-Aug, 13-Aug	01 02	01 02	39.3	NA
FD5	Cuming	Treated	9-Aug, 16-Aug	Pre/Post	Pre/Post	29.1	14 August: 731 ml/ha Lorsban®[2], 365 ml/ha Brigade®[3] (Ground Rig)
FD6	Boone	Treated	14-Aug, 20-Aug	Pre/Post	NA	60.9	17 August: 1462 ml/ha Colbalt®[1](Plane)
FD7	Cuming	Untreated	12-Aug, 19-Aug	01 02	01	13.4	NA
FD8	Cuming	Untreated	12-Aug, 19-Aug	01 02	01	29.3	NA
FD9	Cuming	Untreated	12-Aug, 19-Aug	01 02	01	11.8	NA
FD10	Boone	Treated	14-Aug, 20-Aug	Pre/Post	Pre	99	17 August: 1462 ml/ha Colbalt®[1](Plane)

FD11	Platte	Untreated	16-Aug, 22-Aug	01 02	01	2	NA
FD12	Stanton	Untreated	22-Aug, 26-Aug	01 02	01	60.7	NA

[a]Date which whole plant counts were performed and unbaited Pherocon AM traps were set up.
[b]Foliar insecticide treated fields: Before insecticide application (Pre), after insecticide application (Post). Untreated control fields: First (01) and second (02) time intervals field was sampled.
[c]Date of application, application rate (ml/ha), insecticide, and method of application.
[1]Chlorpyrifos+Lambda-cyhalothrin
[2]Chlorpyrifos
[3]Bifenthrin

Table 2.2. Background: sampling and insecticide application information per field. 2020.

Field	County	Treatment	Sampling Date[a]	WPC[b]	Sticky Trap[b]	Field Size (Ha)	Insecticide(s) Applied[c]
FD2	Colfax	Treated	22-Jul, 29-Jul	Pre/Post	Pre/Post	56.7	27 July: 473.176 ml/ha each, generic chlorpyrifos and bifenthrin (plane)
FD3	Pierce	Treated	NA	Post	NA	32.4	30 July: 443.603 ml/ha Colbalt®[1] (Plane)
FD6	Boone	Treated	4-Aug, 11-Aug	Pre/Post	Pre/Post	60.9	6 August: 473.176 ml/ha Lorsban®[2] (Chemigation)
FD7	Cuming	Untreated	3-Aug, 10-Aug	01 02	01 02	13.4	NA
FD8	Cuming	Untreated	3-Aug, 10-Aug	01 02	01 02	29.3	NA
FD10	Boone	Treated	4-Aug, 11-Aug	Pre/Post	Pre/Post	99	8 August: 473.176 ml/ha Lorsban®[2](Chemigation)
FD12	Stanton	Untreated	11-Aug, 18-Aug	01 02	01 02	60.7	NA
FD15	Saunders	Untreated	7-Aug, 14-Aug	01 02	NA	1.00	NA
FD16	Saunders	Untreated	7-Aug, 14-Aug	01 02	NA	1.00	NA
FD17	Colfax	Treated	22-Jul, 29-Jul	Pre/Post	Pre/Post	56.7	27 July: 473.176 ml/ha each of generic chlorpyrifos and bifenthrin (plane)

[a]Date which whole plant counts were performed and unbaited Pherocon AM traps were set up.
[b]Foliar insecticide treated fields: Before insecticide application (Pre), after insecticide application (Post). Untreated control fields: First (01) and second (02) time intervals field was sampled.
[c]Date of application, application rate (ml/ha), insecticide, and method of application.
[1]Chlorpyrifos+Lambda-cyhalothrin
[2]Chlorpyrifos

Table 2.3. Sex ratio and proportion gravid females obtained from unbaited Pherocon AM traps per field per day 2020; survival to adult stage obtained from emergence cages 2020.

Field	Treatment[a]	Timing[b]	Mean WCR Per Day [c]	Sex ratio (♀/♂)[d]	Total Proportion Gravid[e]	Total Emergence[f]
FD2	T	Time 01	8.9	4.3/4.6	0.4	141
		Time 02	0.10	0.1/0.0	0.1	
FD3	T	NA	NA	NA	NA	68
		NA	NA	NA	NA	
FD5	T	NA	NA	NA	NA	35
		NA	NA	NA	NA	
FD6	T	Pre	4.2	1.1/3.1	0.1	148
		Post	4.1	2.1/2	0.1	
FD7	N	Time 01	14.2	5.9/8.3	0.1	261
		Time 02	20.6	9.0/11.5	0.2	
FD8	N	Time 01	9.6	4.3/5.3	0.1	175
		Time 02	9.4	5.2/4.2	0.1	
FD10	T	Pre	1.4	0.6/0.8	0.1	113
		Post	0.7	0.1/0.5	0.3	
FD12	N	Time 01	18.3	8.7/9.7	0.2	363
		Time 02	45.7	26.1/19.6	0.1	
FD17	T	Pre	5.9	1.5/4.4	0.1	NA
		Post	0.1	0.1/0.0	0	

[a]Treated field (S), untreated (N).

[b]Pre: Sample interval was before insecticide application; Post: Sample interval was after insecticide application. Time 01: First sample interval; Time 02: second sample interval in untreated control fields. All sample intervals were 5-7 days.

[c]Mean western corn rootworms collected per field per day from 12 traps.

[d]Mean male: female western corn rootworm collected per field per day from 12 traps.

[e]Proportion mean western corn rootworm females collected per field that were gravid.
[f]Total western corn rootworms collected from 4 emergence cages per field during the entire year.

Table 2.4. F-test statistics, degrees of freedom, and p-values for model (year, insecticide treatment) and interaction effects on proportion change in mean whole plant counts from time 1 to time 2. 2019, 2020.

Type III Test of Fixed Effects				
Effects	Num DF	DenF	F Value	Pr>F
Year	1	17	0.00	0.9776
Treatment	1	17	317.33	<.0001
Treatment*Year	1	17	0.15	0.6999

Gaussian model, Time was before and after insecticide application in treated fields or time 1 versus time 2 in untreated control fields. Significant effects at P<0.05.

Table 2.5. Proportion change in mean whole plant counts from time 01 to time 02.

2019			**2020**		
Field	**Treatment[a]**	**Proportion Change[b]**	**Field**	**Treatment[a]**	**Proportion Change[b]**
FD1	**T**	**-0.95**	**FD2**	**T**	**-1**
FD2	**N**	-0.2	**FD6**	**T**	-0.78
FD3	**T**	-0.94	**FD7**	**N**	-0.2
FD4	**N**	0.02	**FD8**	**N**	-0.02
FD5	**T**	-1	**FD10**	**T**	-0.88
FD6	**T**	-0.74	**FD12**	**N**	0
FD7	**N**	0.15	**FD15**	**N**	0.02
FD8	**N**	0.04	**FD16**	**N**	0.12
FD9	**N**	0.12	**FD17**	**T**	-0.94
FD10	**T**	-0.98			
FD11	**N**	-0.07			
FD12	**N**	-0.03			

[a]Treatment field (T), untreated field (N).
[b]Proportion change in mean western corn rootworm per plant from Pre to Post spray for insecticide treated fields and Time 01 to Time 02 for control fields.

Table 2.6. F-test statistics, degrees of freedom, and p-values for model (year, time, insecticide treatment) and interaction effects on mean whole plant counts from time 1 to time 2. 2019, 2020.

Type III Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Year	1	13	11.86	0.0044
time	1	13	45.50	<.0001
time*Year	1	13	3.17	0.0983
Treatment	1	13	11.52	0.0048
Treatment*Year	1	13	1.88	0.1938
time*Treatment	1	13	39.18	<.0001
time*Treatment*Year	1	13	3.84	0.0719

Normal distribution, completely random design, split-plot in time, 2x2x2 factorial design. Time was before and after insecticide application in treated fields or time 1 versus time 2 in untreated control fields. Significant effects at P<0.05.

Table 2.7. F-test statistics, degrees of freedom, and p-values for model (year, insecticide treatment) and interaction effects on female: male ratios obtained from unbaited Pherocon AM traps prior to insecticide application.

Type III Tests of Fixed Effects (Female/Male ratio)				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Year	1	15	5.79	0.0295
Insecticide	1	15	0.97	0.3401
Year*Insecticide	1	15	0.37	0.5506

Normal approximation, completely random block design, 2x2 factorial design. Significant effects at P<0.05.

Table 2.8. F-test statistics, degrees of freedom, and p-values for model (year, insecticide treatment)) and interaction effect on proportion gravid females obtained from unbaited Pherocon AM traps prior to insecticide application.

Type III Tests of Fixed Effects (Proportion Gravid)				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Year	1	14.68	49.92	<.0001
Insecticide	1	14.68	0.63	0.4391
Year*Insecticide	1	14.68	0.22	0.6465

Binomial approximation, completely random block design, 2x2 factorial design. Significant effects at P<0.05.

Table 2.9. Sex ratio and proportion gravid females obtained from unbaited Pherocon AM traps per day per field, 2019.

Field	Treatment[a]	Timing[b]	Mean WCR Per Day[c]	Sex ratio (♀:♂)[d]	Proportion Gravid[e]
FD1	T	Pre	2.0	0.6:1.3	0.3
		Post	0.3	0.1:0.1	0.1
FD2	N	Time 01	4.5	1.8:2.6	0.8
		Time 02	3.6	2:1.6	0.7
FD3	T	Pre	0.9	0.5:0.4	0.7
		Post	0.1	0.1:0	0.4
FD4	N	Time 01	7.2	4.3:2.7	0.8
FD5	T	Pre	1.1	0.4:0.7	0.2
		Post	0	0:0	0
FD7	N	Time 01	2.0	1.4:0.6	0.8
FD8	N	Time 01	2.5	1:1.4	0.8
FD9	N	Time 01	2.5	1.0:1.5	0.9
FD10	T	Pre	9.4	6.0:3.4	0.7
FD11	N	Time 01	28.9	15.6:13.2	0.7
FD12	N	Time 01	7.6	5.1:2.5	0.5

[a]Treated field(S), untreated (N).
[b]Pre: Sample interval was before insecticide application; Post: Sample interval was after insecticide application. Time 01: First sample interval; Time 02: second sample interval in untreated control fields. All sample intervals were 5-7 days.
[c]Mean western corn rootworms collected per day per field from 12 traps.
[d]Mean male: female western corn rootworm collected per day per field from 12 traps.
[e]Proportion mean western corn rootworm females collected per field that were gravid.

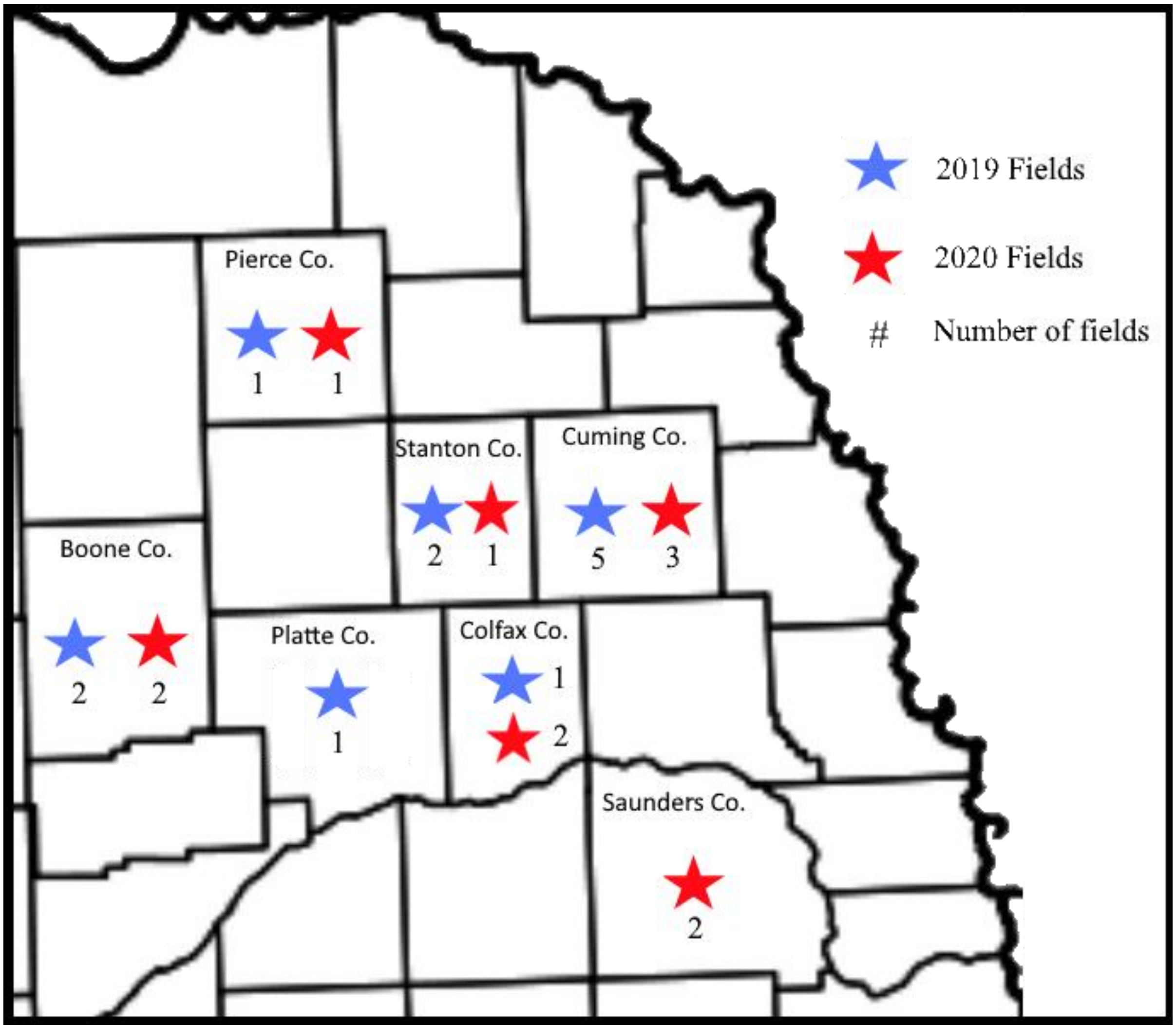


Figure 2.1. County map of northeast Nebraska. Blue and red stars indicate fields were sampled in 2019 or 2020, respectively. The number of fields sampled per year and county is listed under each star.

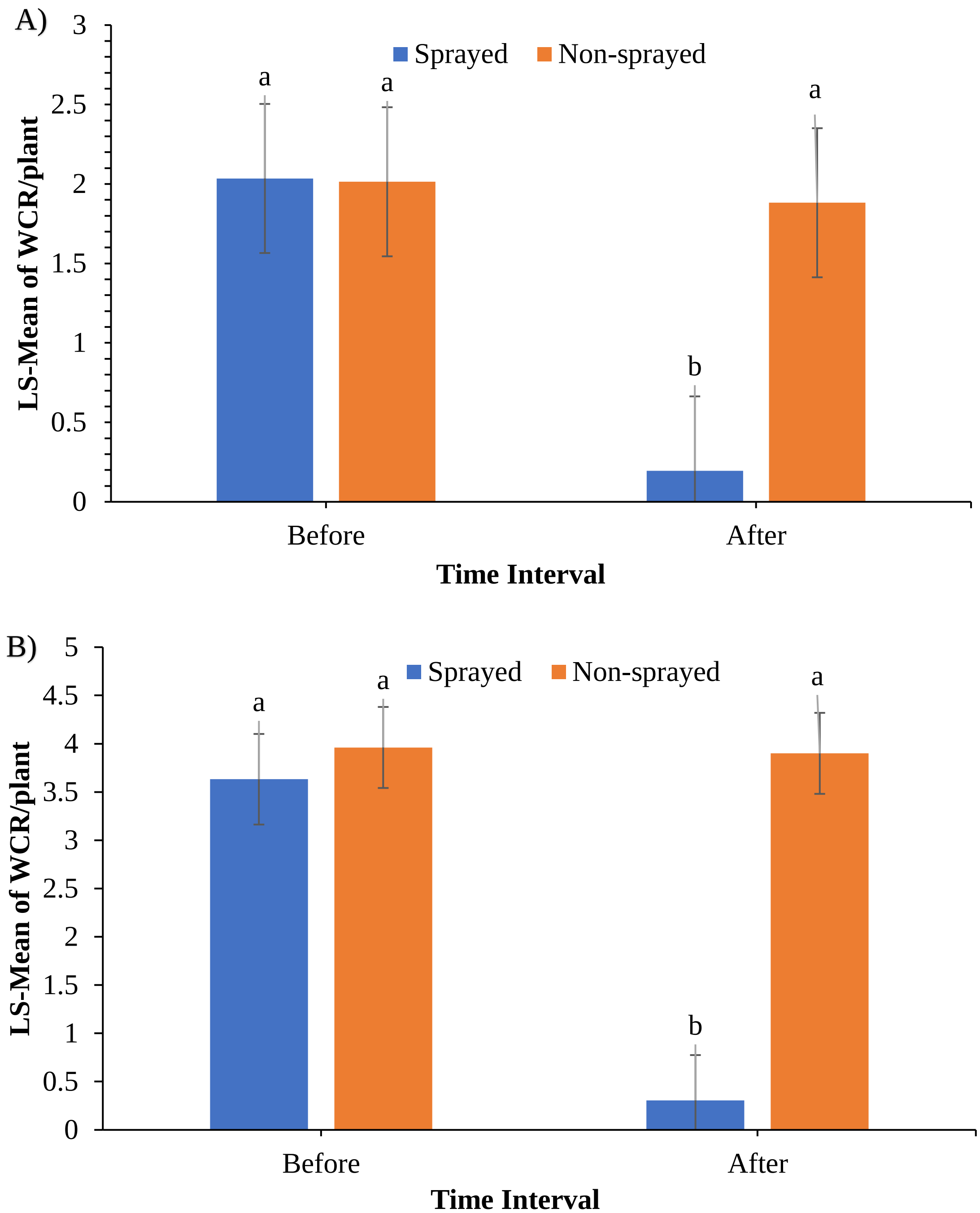


Figure 2.2. Least-Square Mean±SE estimates of treatment fields by time (WCR/plant) A) LS-Means of 2019 sprayed and non-sprayed fields. B) LS-Means of 2020 sprayed and non-sprayed fields. Bars with the same lower-case letter are not significantly different (*P*>0.05).

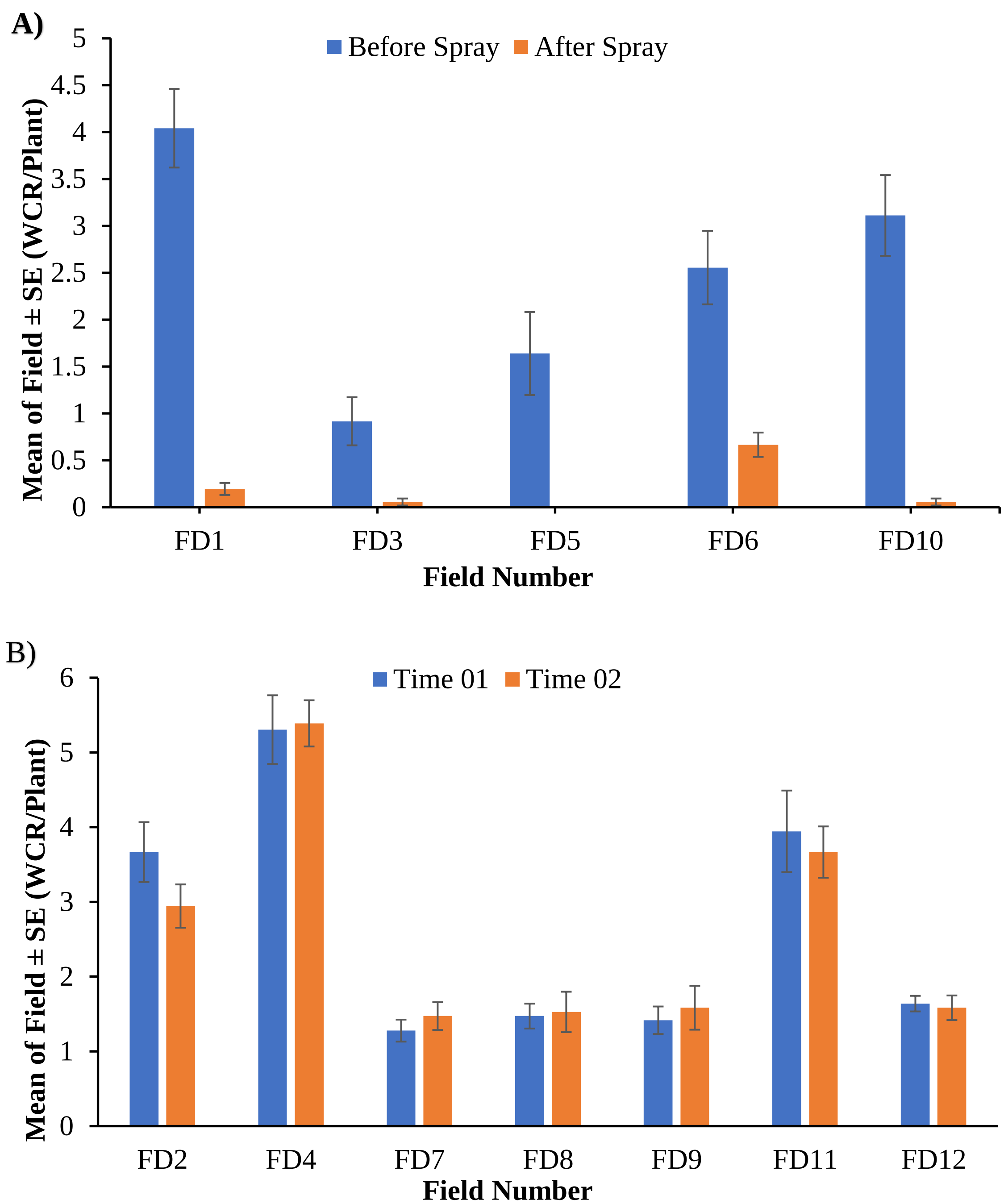


Figure 2.3. Mean western corn rootworm per plant counts from fields sampled in 2019. A) Foliar insecticide treated fields before and after application, B) Untreated fields during time period 01 followed by time period 02. Individual bars represent mean ± standard error.

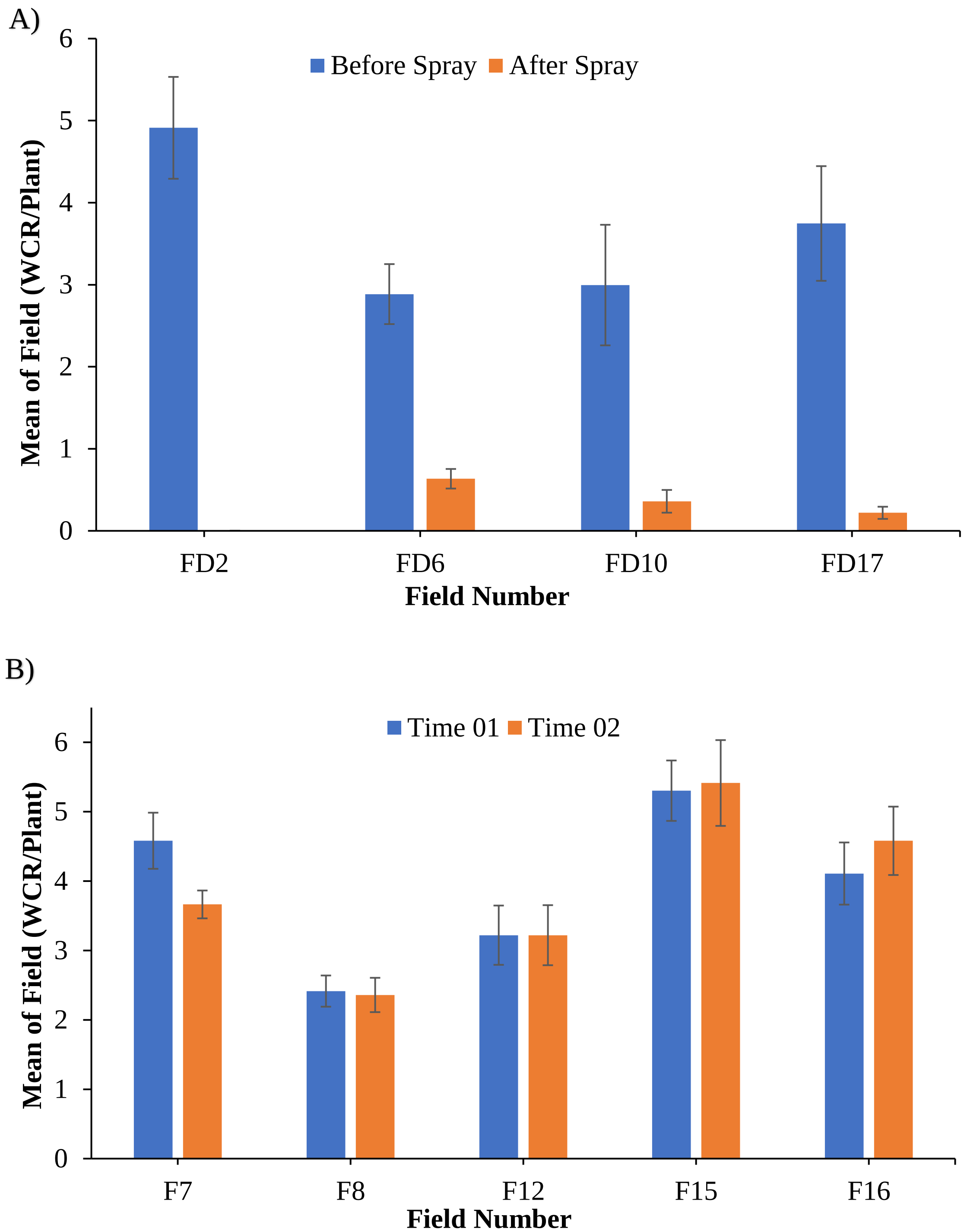


Figure 2.4. Mean western corn rootworm per plant counts from fields sampled in 2020. A) Foliar insecticide treated fields before and after application, B) Untreated fields during time period 01 followed by time period 02. Individual bars represent mean ± standard error.

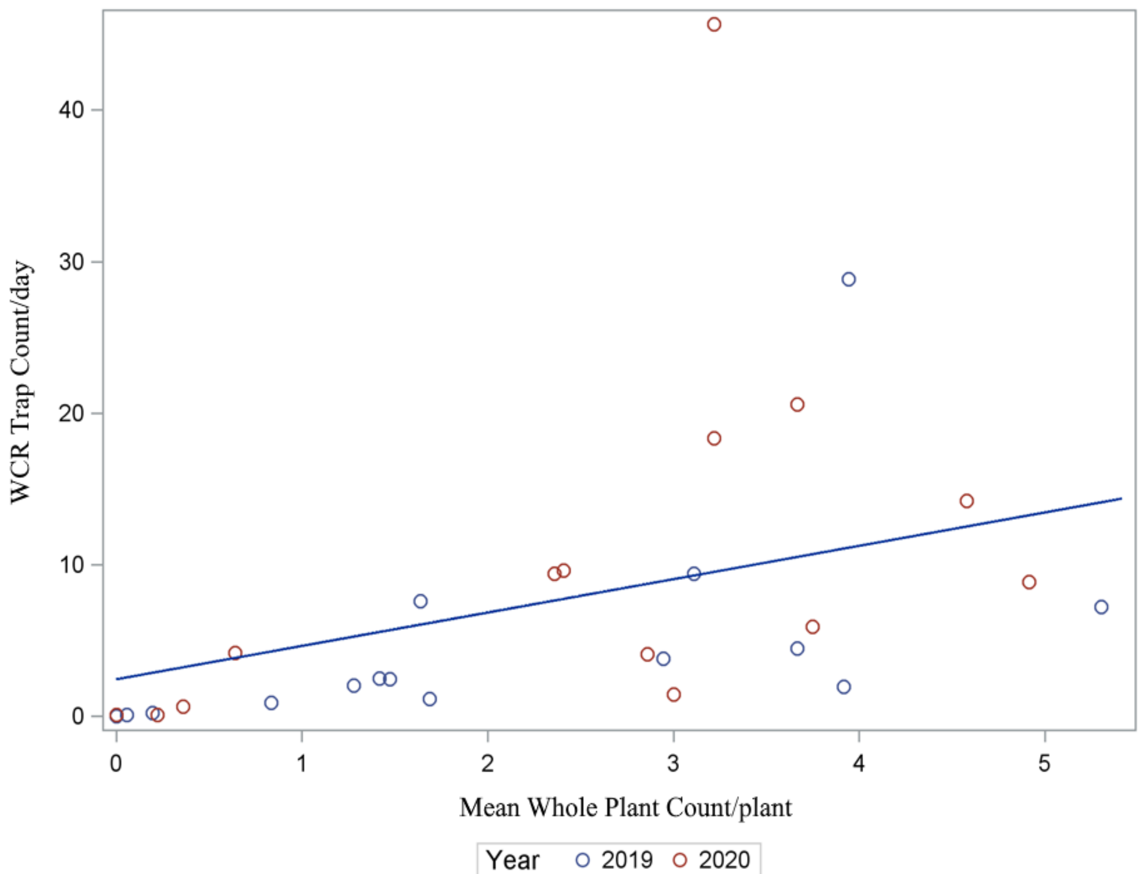


Figure 2.5. Relationship between mean western corn rootworm whole plant counts per field and mean western corn rootworm collected on unbaited Pherocon AM sticky traps per field per day. Each dot represents a separate mean whole plant count in an individual field and the 5–7-day trap sampling interval directly following the date the whole plant count was recorded. Blue circles: 2019 fields. Red circles: 2020 fields. The blue line represents the estimated regression equation.

Chapter 3: SUSCEPTIBILIY OF WESTERN CORN ROOTWORM TO ACTIVE INGREDIENTS: BIFENTHRIN, CHLORPYRIFOS, AND DIMETHOATE

3.1. Introduction

The western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte, (Coleoptera: Chrysomelidae) is a major economic pest of maize, *Zea mays* L., in the United States and has historically adapted relatively quickly to management tactics (Gray et al. 2009, Miller et al. 2009, Wechsler and Smith 2018, Gassmann 2021, Meinke et al. 2021). WCR has especially shown rapid resistance evolution to selection with the chemical control tactics soil and foliar insecticides (Ball and Weekman 1962, Meinke et al. 1998, Pereira et al. 2015, Souza et al. 2019, Meinke et al. 2021).

Soil insecticides can be applied either as granular or liquid applications in maize production systems. They were first introduced as a control tactic in the 1940's and field-evolved WCR resistance to broadcast-applied organochlorine and cyclodiene compounds was documented a decade later in Nebraska (Roselle et al. 1959, Ball and Weekman 1962, 1963, Chio et al 1978). Due to widespread WCR resistance to cyclodienes, several alternative management options emerged. Organophosphate (OP) and carbamate insecticide classes replaced the cyclodienes as soil insecticides but primarily due to increased cost were only applied in-furrow or banded over the row (Mayo 1986). This provided a natural untreated refuge between rows which has likely prevented WCR from evolving resistance to these insecticides when deployed in this way (Souza et al. 2019). A second alternative was the use of foliar insecticides that target adult WCR to reduce densities and subsequent larval injury the following season (Pruess et al. 1974, Meinke 1995, Meinke et al. 2021). Organophosphate and carbamate foliar applications were initially effective at managing WCR densities, but extensive use eventually produced resistant adults and control failures in south-central Nebraska (Meinke et al. 1998, Miota et al. 1998, Scharf et al. 1999, Parimi et al. 2006).

Transgenic maize expressing insecticidal proteins derived from *Bacillus thuringiensis* Berliner (Bt), was introduced in the 2000s, was widely adopted, and replaced soil insecticides as the primary tactic used to manage WCR (Rice 2004, Andow et al. 2016, Meinke et al. 2021). Since 2009, field-evolved resistance to Bt-traits has been documented in local areas of the U.S. Corn Belt (Gassmann et al. 2011, Wangila et al. 2015, Zukoff et al. 2016, Ludwick et al, 2017) which has increased insecticide usage to complement other tactics and mitigate WCR resistance to transgenic maize.

During the 1990s, many carbamates and OPs were removed from the market by regulatory action leaving primarily pyrethroids and a few OPs to manage WCR. In Nebraska alone, there was a 40% increase in bifenthrin use by 2014 (NASS-USDA). This included soil and foliar applications for WCR control, and foliar applications targeting secondary pests (i.e., spider mites *Tetranychus urticae* Koch and western bean cutworm *Striacosta albicosta* Smith (Pereira et al. 2015, 2017, Souza et al. 2019). In this system, WCR was exposed annually to bifenthrin both as a target or nontarget insect. The selection pressure imposed from one or more aerial applications of bifenthrin per year led to WCR field-evolved resistance in Kansas and Nebraska (Pereira et al. 2015, 2017, Souza et al. 2019). Dimethoate bioassays also revealed a low level of resistance in southwest Nebraska (Souza et al. 2019).

Chlorpyrifos, an organophosphate, has been used as both a soil insecticide and foliar insecticide to manage WCR. There has been extensive documentation of formulated chlorpyrifos efficacy as a soil insecticide (e.g., DeVries and Wright 2002a, 2002b, Calvin et al. 2004a 2004b, Dana et al. 2013) but less data is available on foliar chlorpyrifos efficacy when targeting adults (Zhu et al. 2005, Tinsley et al. 2014a, 2014b). Currently, both generic chlorpyrifos and pyrethroids have been commonly used for adult WCR control in Nebraska because of relatively low cost. A recent field study that evaluated the efficacy of commercial application of formulated chlorpyrifos and the pyrethroids bifenthrin or lambda-cyhalothrin in northeast Nebraska revealed that both insecticide classes provided excellent control of adult WCR with some exceptions (Chapter 2).

Therefore, this study was conducted as a companion to the field study to develop active ingredient concentration-response data from some populations included in the field study to further characterize current WCR susceptibility levels in northeast Nebraska as a baseline for future research and integrated management programs (IPM). WCR were bioassayed with bifenthrin, chlorpyrifos, and dimethoate active ingredients.

3.2. Materials and Methods

3.2.1. Western Corn Rootworm Populations

Eleven WCR laboratory populations were established from populations collected from 6 different counties throughout northeast Nebraska (Table 3.1). Beetles were collected during July-August in 2019 and 2020 prior to insecticide application. The Boone Co. field FD6, after foliar insecticide application, had persistently high WCR densities so a second collection was made to further investigate susceptibility. Therefore, field FD6 is represented by 2 adult populations, before (FD6-Pre) and after (FD6-Post) application.

A minimum of 50 gravid females, but most collections >150 gravid females, were collected per field to obtain an adequate representation of the natural genetic variation present. Adults recovered from these counties were delivered to the University of Nebraska-Lincoln on the day of collection and maintained in plexiglass cages (28 cm^3). Procedures used to maintain adults, collect, and process eggs, and temperature regimes to facilitate diapause and post-diapause development are described in Wangila et al. (2015) and Reinders et al. (2018).

3.2.2. Selected Bifenthrin Colony

A selected laboratory WCR colony (bif-R. population) with a history of long-term exposure to bifenthrin was bioassayed to determine preservation or regression of susceptibility after cessation of bifenthrin exposure. The colony was originally collected and established in 2014 from Perkins Co., NE from a field with a history of continuous maize plus annual soil- and aerial application of bifenthrin for at least five consecutive years (Souza et al. 2019). The bif-R. population was reared in non-diapause background for 9 generations under adult selection with a diagnostic concentration (LC_{99}) of bifenthrin (Pereira et al. 2015, Souza et al. 2019) and then maintained in the laboratory without bifenthrin selection through generation 26. Adults from generation 26 were bioassayed in this study with bifenthrin during 2021.

3.2.3. Control Population

A non-diapause western corn rootworm population originated from Crop Characteristics (C.C.), Inc. in Farmington, MN was used as the susceptible control for all bioassays. Shipments of non-diapausing male and female beetles, were received from C.C. and reared in plexiglass cages (28 cm^3) for 24 hours before initiating laboratory experiments.

3.2.4. Adult Vial Insecticide Bioassays

Insecticide Active Ingredients: Three active ingredient insecticide treatments were tested: the organophosphate chlorpyrifos (chlorpyrifos 99.5% purity, Cat. No. N-11459), the organophosphate dimethoate (dimethoate 99.4% purity, Cat. No. N-11758), and the pyrethroid bifenthrin (bifenthrin 96.7% purity, Cat. No. N-11203). All insecticides were purchased from Chem Service Inc. (West Chester, PA). Each insecticide was dissolved in acetone ≥ 99.5% purchased from Sigma-Aldrich Corp. (St. Louis, MO) for stock solution preparation.

Serial Dilution: Vials coated with insecticide were prepared via serial dilution diluted with acetone. A serial dilution is a series of sequential dilutions to convert a highly concentrated solution into a management concentration for experimentation (Sapkota 2020), this involved doubling the initial amount of solution to continuously dilute the solution in decreasing concentration to the desired concentration range. The concentration of the next dilution will always be half the previous concentration (i.e. 8.0, 4.0, 2.0 µl/vial, etc.). A new stock solution of insecticide was prepared beforehand for each serial dilution performed.

Stock solution formula was (volume of insecticide g) x 1000 µl acetone mixed in a centrifuge tube. Serial dilution calculations consisted of:

$$\mathrm{X}_1 = \frac{\text{Highest Insecticide Conc. x repetitions x 2}}{\text{Insecticide purity}}$$

$$x = \frac{\mathrm{X}_1(1000\mu\mathrm{l})}{(\text{volume of insecticide } \mu\text{g})}$$

Where x is the amount of insecticide solution needed from the prepared stock solution. Acetone for dilution followed a similar formula:

$$Total\ Acetone = \text{ 500 µL/vial of Acetone x number of trials/repetitions x 2.}$$

Total acetone was subtracted by the volume of insecticide for the actual volume acetone needed for the serial dilution.

Vial Bioassay: The WCR bioassays were conducted at the University of Nebraska-Lincoln. The susceptibility of adult WCR to insecticide active ingredients was estimated in 2020 when first-generation progeny of beetles collected in 2019 emerged. Additional bioassays of adult WCR were conducted in 2021 with first-generation progeny from collections made in 2020 with insecticides not tested in 2020. The selected bif-R lab colony was bioasayed when generation 26 emerged.

The vial bioassay procedures for adult WCR of Scharf et al. (1999) optimized by Souza et al. (2019) were used. Wheaton™ Glass 20 mL scintillation vials (Thermo Fisher Scientific Inc., Waltham, MA, Cat. No. 03-340-25N) were treated with 500 µl of increasing concentrations of chlorpyrifos, bifenthrin, or dimethoate diluted with acetone. A negative control, a vial treated with 500 µl acetone only, was included with each vial bioassay. The concentration (conc.) range for each insecticide applied on each WCR population varied from 6-10 concentrations depending on insecticide and susceptibility to obtain a complete concentration-response curve. Three repetitions per population were completed for each tested insecticide. Vials were coated with insecticide solution homogenously and allowed to dry under a fume hood for 30 min. at room temperature on a commercial roller machine (Nemco 8045SXW Hot Dog Roller Grill, Nemco Food Equipment Inc., Hicksville, OH).

Each treatment vial was infested with 10, 48hr to 72hr-old adults of even sex-ratio. Vial caps were loosely closed to facilitate air exchange and prevent beetles from escaping. After 24hrs of infestation, beetle mortality was recorded. One kernel of sweet corn was added to each vial to allow beetle feeding after 24hrs post-infestation, and at 48hrs, beetle mortality was again recorded. Beetles that did not respond to prodding, unable to walk when flipped, and twitched only when prodded were pronounced dead. Preliminary results with a cross-section of populations indicated that percent mortality either did not significantly change or increased with exposure duration, so 24hr was used as the final bioassay duration for each active ingredient.

3.3. Statistical Analysis

Vial Bioassay. The susceptibility of adult WCR populations to insecticide active ingredient was assessed by analyzing the relationship between insecticide concentration and adult mortality. The percent mortality of each population to insecticide concentrations was used to estimate LC_{50} by probit analysis using POLOPlus-PC software (LeOra Software LLC) (Finney 1971, Russell and Robertson 1979, LeOra 1987). The data were corrected by Abbot’s formula for natural control mortality and analyzed with a probit link function with Normal distribution. The statistical analysis also estimated a Pearson goodness-of-fit chi-square value ($\chi 2$) testing the null hypothesis that the regression model adequately fit the data. Resistance ratios (RR_{50}) with corresponding 95% confidence intervals (95%CI) were calculated by dividing the estimated LC_{50}s and 95% CI of resistant populations by the estimated LC_{50} and 95% CI of the control population (Robertson et al. 2017).

3.4. Results

3.4.1. WCR Bifenthrin Bioassays

All populations tested with bifenthrin susceptibility was similar to the control non-diapause population (control) based on LC_{50}s 95% confidence interval (CI) overlap, except for WCR from field FD6 and the bif-R selected lab colony (Table 3.2). LC_{50} dose ranged from 0.19 µg/vial (FD12 population) to 2.73 µg/vial (FD6 population post-application). The CIs of FD6-Pre and FD6-Post LC_{50}s overlapped, and the slopes were similar (Table 3.2) (parallel in the tests of equality ($\chi^2 = 0.33$, $p>0.05$) and parallelism ($\chi^2 = 0.00$, $p>0.05$) of slopes and intercepts). The resistance ratios (RR) of FD6-Pre and FD6-Post were 6- and 7-fold, respectively, for bifenthrin with overlapping CIs which matches the LC_{50} CI overlap and supports the equality and parallelism results that the two populations were similar only differing in treatment (Table 3.2).

Two clusters of bifenthrin susceptibility were apparent when field populations were compared (Fig. 3.1). The populations from FD3, FD6, and FD10 were the most tolerant to bifenthrin with overlapping LC_{50} CIs but the RRs of FD6-Pre and FD6-Post populations were significantly greater than those recorded for FD3 and FD10 populations

(Table 3.2). The slope of FD10 was close to FD6s but was significantly different (χ^2= 11.96, p<0.05), but parallel (χ^2= 0.87, p>0.05) by the tests of equality and parallelism, respectively. The slope of FD3 was significantly different from the other two populations.

The populations from FD5, FD8, FD12, and FD16 were the most susceptible to bifenthrin. All had overlapping LC_{50} CIs (Table 3.2). The slopes of FD8 and FD12 were found to be significantly different (χ^2= 18.51, p<0.05) but parallel (χ^2= 1.79, p>0.05), while the slopes of FD5 and FD16 were significantly different from each other and FD8 and FD12 (Table 3.2).

3.4.2. WCR Chlorpyrifos Bioassays

The populations exhibited variable levels of susceptibility to chlorpyrifos, but based on 95% CI overlap of LC_{50}s, with the exception of WCR from FD1, all populations were significantly more tolerant to chlorpyrifos than the control (Table 3.3). LC_{50s}, ranged from 1.16 μg/vial (FD12) to 3.72 μg/vial (FD6-Post population), and RR, ranged from 1.62- 5.22-fold (Table 3.3). The FD6-post population exhibited the highest $LC_{50,}$ but 95% CI overlapped with populations from fields 1, 2, and 17 (Table 3.3). However, the FD6-Post population had the highest RR, and the CI did not overlap with any other population suggesting that FD6-post was the most tolerant to chlorpyrifos (Table 3.3). In general, there was a lot of overlap in CIs of LC_{50}s and RRs of field collections as populations from FD1, FD2, FD3, FD5, FD6-Pre, FD10, and FD17 were not significantly different (Table 3.3, Fig. 3.2).

When comparing susceptibility of the two WCR cohorts from FD6, the WCR population from FD6-pre was significantly different than the population post treatment, with nonoverlapping LC_{50} and RR CI (Table 3.3; Fig. 3.2). Comparing the slopes of all field populations further differentiated the two F6 cohorts and revealed the similarity between the FD10 population slope and FD6-Post population slope (parallelism (χ^2= 0.38, $p>0.05$) (Table 3.3). No significant interaction between the two populations was found (test of equality (χ^2= 18.06, $p<0.05$).

3.4.3. WCR Dimethoate Bioassays

The two field populations bioassayed with dimethoate, FD1 and FD6-Pre had similar LC_{50}, RR, overlapping CI, and were as susceptible as the control population (Table 3.2, Fig. 3.3). The tests of equality and parallelism indicated no significant differences between the two populations (Equal: χ^2= 2.82, $p>0.05$; Parallel: χ^2= 2.78, $p>0.05$).

3.5. Discussion

The vial bioassay results indicate a mosaic of WCR susceptibility to bifenthrin and chlorpyrifos occurs in northeast NE with many populations relatively susceptible to each active ingredient. Results are similar to those reported from a topical assay study in which WCR populations were susceptible to bifenthrin in eastern NE (Meinke et al. 1998). Populations from Cuming and Pierce Co., northeast NE, previously bioassayed with a diagnostic bifenthrin dose (LC_{99}) had 59-88% mortality indicating that there were resistant alleles in some populations (Pereira et al. 2015, 2017). This is in contrast to susceptible FD5 and FD8 populations from Cuming Co. in this study which had LC_{50} and RR similar to the control population. Bioassay data track very well with the level of control obtained with foliar-applied formulated products using the same field populations

(Table 3.2, 3.3; chapter 2). This was especially apparent with FD6 which exhibited elevated LC_{50} values in lab bioassays and the lowest level of control with formulated products in the field (Table 3.2, 3.3; chapter 2). In contrast, fields where WCR control with formulated products was excellent were found to be very susceptible in the active ingredient bioassays (Table 3.2, 3.3; chapter 2).

The bifenthrin susceptible and more tolerant clusters of populations revealed by lab vial bioassays correlate well with known past exposure to foliar-applied pyrethroids (Tables 3.2, 3.4). The more bifenthrin-tolerant populations had been exposed to either formulated bifenthrin or lambda-cyhalothrin in 3 of 4 years from 2017-2020 while the more susceptible cluster of populations had no exposure to foliar-applied pyrethroids during the same period. The two cohorts from FD6 had elevated LC50s and 6-7 fold RRs indicating reduced susceptibility to bifenthrin. The FD10 population also displayed reduced susceptibility, although not to the level displayed by the FD6 population (Table 3.2; Fig. 3.1). The flat slopes of FD6 and FD10 populations plus wide LC_{50} CIs indicate that they were highly heterogeneous in toxicological response with susceptible and highly resistant individuals present within the populations. The FD6 adult RRs are also similar to those reported by Pereira et al. (2015) when emerging resistance to bifenthrin was first confirmed in southwestern NE. Therefore, these factors collectively suggest that field-evolved resistance to bifenthrin is occurring in WCR populations bioassayed from Boone Co.

The selected bif-R lab population was confirmed in lab bioassays to be highly resistant to bifenthrin (Souza et al. 2019). Souza et al. (2019) reported LC_{50s} as high as 10.5 μg/vial and RR 40- to 55- fold RRs whereas the results of this study revealed a much lower LC_{50} and only a 2.3-fold RR (Table 3.2). This indicates that resistance in the bifenthrin population regressed to a more susceptible state in absence of continued bifenthrin insecticide exposure. This is an interesting case of regression as the WCR has a history of maintaining resistance alleles to cyclodiene and organophosphate insecticides with minimal fitness costs in the absence of selection pressure (Parimi et al. 2006). A reason for this regression is unclear and could simply be due to genetic bottlenecks after the bifenthrin selection experiments, reducing genetic variation through inbreeding after 26 generations. Other scenarios are possible such as a possible role of epigenetics (Brevik et al. 2018). Resistance alleles may have only been induced/expressed when selection pressure from bifenthrin was present. More research is needed on the bif-R population to clarify the reason for the decline in resistance.

Souza et al. (2019) extended the bifenthrin bioassay duration from 24- to 48hr because adult survival increased over time in some populations. This trend was not observed in this study. The difference may have been due to the level of bifenthrin resistance present in each study. The 48hr time period was necessary in Souza et al. (2019) experiments because resistance alleles appeared to allow some beetles to metabolize the toxin within a 24hr time interval and recover. The low level of resistance displayed in this study was probably not enough to allow beetles to overcome the effects of the toxin, thus no difference between 24hr and 48hr survival.

The chlorpyrifos bioassays revealed most populations tested were similar in susceptibility (RR <3.6 fold) with the exception of FD6-post with significantly greater LC_{50} and RR (5.2-fold) than other populations. The FD6 WCR population collected before insecticide application displayed a significantly lower LC_{50} and RR than the FD6-

Post population, a different trend than observed in the bifenthrin bioassays (Table 3.2, 3.3) (Fig. 3.2, 3.3). This provides strong evidence that the FD6 population contained resistant and susceptible alleles and that the formulated insecticide applications applied in the previous field study were placing selection pressure on the population, potentially shifting the population to an early stage of resistance evolution. Formulated chlorpyrifos+lambda-cyhalothrin (2019) or chlorpyrifos (2020) in the companion field study provided the lowest level of control (74-78% control, Chapter 2) which tracks well with the F6 bifenthrin and chlorpyrifos bioassay results.

With the exception of the cyclodienes, where RRs of >1000 were recorded (Siegfried and Mullen 1989, Parimi et al. 2006), it is common for relatively low WCR RRs to be associated with reduced efficacy or measurable crop injury in the field after selection over time with a toxin in the field. In south-central NE, after long-term use of adult management programs, WCR field-evolved resistance to methyl parathion and carbaryl occurred during the 1990s (Meinke et al. 1998, Miota et al.1998). Topical assays identified relatively low RRs (methyl parathion: 16.4-fold, carbaryl: 9.1-fold) which were enough to make the adult management program ineffective (Meinke et al. 1998). The previously discussed WCR 5- to 10-fold RR to bifenthrin was associated with noticeable reduction in field efficacy in southwestern NE (Pereira et al. 2015, 2017). The RRs associated with WCR field-evolved resistance to the Bt protein Cry3Bb1 were initially only 3-6 fold but were great enough to facilitate severe crop damage in the field (Gassmann et al. 2011). The FD6 bifenthrin and chlorpyrifos data from this study provide another example of low WCR RRs associated with measurable reduction of efficacy in the field (chapter 2).

Chlorpyrifos is not cross-resistant to methyl-parathion and methyl-parathion resistance was not historically detected in northeast Nebraska, so it is unlikely that there is a relationship between the organophosphates affecting susceptibility in the area of this study (Meinke et al. 1998, Miota et al. 1998, Wright et al. 2000). But cross-resistance between chlorpyrifos and bifenthrin may be possible because the mechanisms of resistance of pyrethroids, carbamates, and OPs can be similar: 1) they share structurally similar ester bonds that are susceptible to hydrolysis of esterases, and 2) metabolism of toxin by enhanced expression of cytochrome P450 (Miota et al. 1998, Scharf et al. 2000, Souza et al. 2020a, 2020b). Populations that exhibited elevated LC_{50}s for methyl-parathion and carbaryl also had elevated bifenthrin LC_{50}s which suggested increased enzyme activity after years of exposure may have affected each other (Meinke et al. 1998). This may be the case for some populations in this study with historic treatment of both insecticides and/or tank-mixes of both contributing to the differences from the control population (Chapter 2) (Table 3.4).

Only a few populations were bioassayed with dimethoate, but from the preliminary data, it appears that Cuming and Boone Co. were relatively susceptible to the OP dimethoate (Table 3.2, Fig. 3.3). In contrast, previous bioassays by Souza et al. (2019) revealed a low level of resistance to dimethoate (3- to 16- fold RR) for a southwest Nebraska population. Dimethoate and indoxacarb which exhibits negative cross-resistance when pyrethroid resistance is present could be useful insecticide alternatives in areas where resistance is emerging to pyrethroids (Souza et al. 2019).

In summary, results from active ingredient bioassays indicate a mosaic of WCR susceptibility to pyrethroid and organophosphate insecticides exists in northeast NE.

Most populations bioassayed were susceptible (most < 2-3.6 RR) but we conclude that a low level of resistance to bifenthrin and chlorpyrifos (5-7 fold RR) is evolving in a few populations. The past formulated product field history and efficacy recorded in the companion formulated product field study were highly correlated with active ingredient bioassay data. This study provides a good baseline of current susceptibility to commonly used insecticide in northeastern NE that will inform future studies and current IRM/IPM programs. Additional data is needed from areas between northeastern and southwestern NE to gain a more complete picture of WCR susceptibility to insecticides in NE.

Reference Cited

Andow, D. A., S. G. Pueppke, A. W. Schaafsma, A. J. Gassmann, T. W. Sappington, L. J. Meinke, P. D. Mitchell, T. M. Hurley, R. L. Hellmich, and R. P. Porter. **2016**. Early detection and mitigation of resistance to Bt maize by western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 109: 1–12.

Ball, H. J., and G. T. Weekman. **1962**. Insecticide resistance in the adult western corn rootworm in nebraska. J. Econ. Entomol. 55: 439–441.

Ball, H. J., and G. T. Weekman. 1963. Differential resistance of corn rootworms to insecticides in Nebraska and adjoining states. J. Econ. Entomol. 56:553–555.

Brevik, K., et al. 2018. Transgenerational effects of insecticides-implications for rapid pest evolution in agroecosystems. Curr. Opin. Insect Sci. 26: 34-40.

Calvin, D. D., L. Smith, and P. R. Rebarchak. **2004a**. Corn rootworm larval control, 1999. Arthropod Manag. Tests. 29. doi.org/10.1093/amt/29.1.F17

Calvin, D. D., S. Sked, and S. Smiles. **2004b**. Corn rootworm larval control, 2002. Arthropod Manag. Tests. 29. doi.org/10.1093/amt/29.1.F19

Chandler, L. D. 2003. Corn rootworm areawide management program: United States Department of Agriculture-Agricultural Research Service. Pest Manag. Sci. 59: 605–608.

Chio H., C. C. Chang, R. L. Metcalf, and J. Shaw. 1978. Susceptibility of four species of *Diabrotica* to insecticides. J. Econ. Entomol. 71:389–393.

Dana, L. A., T. E. Hunt, and K. Jarvi. **2013**. Control of rootworm larvae in corn, 2012. Arthropod Manag. Tests. 38. doi.org/10.4182/amt.2013.F8

DeVries, T., and R. J. Wright. **2002~~a~~.** Larval corn rootworm control, 2001A. Arthropod Manag. Tests. 27. doi.org/10.1093/amt/27.1.F15

DeVries, T., R. J. Wright, L. Meinke, and B. Siegfried. **2002~~b~~**. Evaluation of soil insecticides for control of resistant corn rootworm, 2001. Arthropod Manag. Tests. 27. doi.org/10.1093/amt/28.1.F11

Finney, D. J. 1971. Probit analysis, 3rd ed. Cambridge University Press, Cambridge.

Gassmann, A. J. 2021. Resistance to Bt maize by western corn rootworm: effects of pest biology, the pest–crop interaction and the agricultural landscape on resistance. Insects. 12: 136.

Gassmann, A. J., J. L. Petzold-Maxwell, R. S. Keweshan, and M. W. Dunbar. 2011. Field-evolved resistance to Bt maize by western corn rootworm. PLoS ONE 6: e22629.

Gray, M. E., T. W. Sappington, N. J. Miller, J. Moeser, and M. O. Bohn. **2009**. Adaptation and invasiveness of western corn rootworm: intensifying research on a worsening pest. Annu. Rev. Entomol. 54: 303–321.

LeOra. 1987. Polo-PC: a user's guide to probit or logit analysis. LeOra Software, Berkeley.

Ludwick, D. C., L. N. Meihls, K. R. Ostlie, B. D. Potter, L. French, and B. E. Hibbard. 2017. Minnesota field population of western corn rootworm (Coleoptera: Chrysomelidae) shows incomplete resistance to Cry34Ab1/Cry35Ab1 and Cry3Bb1. J. Appl. Entomol. 141: 28-40.

Mayo, Z. B. 1986. Field evaluation of insecticides for control of larvae of corn rootworms, pp. 183-203. Methods for the Study of Pest Diabrotica. Springer Series in Experimental Entomology. Springer, New York, NE

Meinke, L. J. 1995. Adult corn rootworm management. MP63-C, Agricultural Research Division, Institute of Agriculture and Natural Resources. University of Nebraska, Lincoln, Nebraska.

Meinke, L. J., B. D. Siegfried, R. J. Wright, and L. D. Chandler. 1998. Adult susceptibility of Nebraska western corn rootworm (Coleoptera: Chrysomelidae) populations to selected insecticides. J. Econ. Entomol. 91: 594-600.

Meinke, L. J., D. Souza, and B. D. Siegfried. 2021. The use of insecticides to manage the western corn rootworm, *Diabrotica virgifera virgifera*, LeConte: history, field-evolved resistance, and associated mechanisms. Insects. 12:112. DOI: 10.3390/insects12020112.

Miller, N. J., T. Guillemaud, R. Giordano, B. D. Siegfried, M. E. Gray, L. J. Meinke and T. W. Sappington. **2009**. Genes, gene flow and adaptation of *Diabrotica virgifera virgifera*. Agric. For. Entomol. 11: 47–60.

Miota, F., M. E. Scharf, M. Ono, P. Marçon, L. J. Meinke, R. J. Wright, L. D. Chandler, and B. D. Siegfried. **1998**. Mechanisms of methyl and ethyl parathion resistance in the western corn rootworm (Coleoptera: Chrysomelidae). Pestic. Biochem. Physiol. 61: 39–52.

NASS-USDA, USDA/NASS QuickStats Ad-hoc Query Tool. *[Online]. Available:* https://quickstats.nass.usda.gov/ *[30 June 2021].*

Parimi, S., L. J. Meinke, B. Wade French, L. D. Chandler, and B. D. Siegfried. **2006**. Stability and persistence of aldrin and methyl-parathion resistance in western corn rootworm populations (Coleoptera: Chrysomelidae). Crop Protection. 25: 269–274.

Peters, L. L. and S. R. Lowry. 1991. Western corn rootworm (Coleoptera: Chrysomelidae) larval control with chlorpyrifos applied at planting time versus a post-planting chemigation application to corn grown under two different tillage systems. J. Kans. Entomol. Soc. 64: 451-454.

Pereira, A. E., D. Souza, S. N. Zukoff, L. J. Meinke, and B. D. Siegfried. **2017**. Cross-resistance and synergism bioassays suggest multiple mechanisms of pyrethroid resistance in western corn rootworm populations. PLoS ONE 12(6): e0179311. https://doi.org/10.1371/journal.pone.0179311

Pereira, A. E., H. Wang, S. N. Zukoff, L. J. Meinke, B. W. French, and B. D. Siegfried. 2015. Evidence of field-evolved resistance to bifenthrin in western corn rootworm (Diabrotica virgifera virgifera LeConte) populations in western Nebraska and Kansas. PLoS ONE. 10(11): e0142299. doi:10.1371/journal.pone.0142299.

Pruess, K. P., J. F. Witkowski, and E. S. Raun. **1974**. Population suppression of western corn rootworm by adult control with ULV malathion. J. Econ. Entomol. 67: 651–655.

Reinders, J. D., B. D. Hitt, W. W. Stroup, B. W. French, and L. J. Meinke. 2018. Spatial variation in western corn rootworm (Coleoptera: Chrysomelidae) susceptibility to Cry3 toxins in Nebraska. PLoS ONE 13: e0208266.

Rice, M. E. 2004. Transgenic rootworm corn: assessing potential agronomic, economic, and environmental benefits. Plant Health Prog. doi: 10.1094/PHP-2004-0301-01-RV. (http://www.plantmanagementnetwork.org/sub/php/review/2004/rootworm/)

Robertson, J. L., M. M. Jones, E. Olguin, and B. Alberts. **2017**. Bioassays with Arthropods, 3rd ed. Chapman and Hall, CRC, London.

Roselle, R. E., L. W. Anderson, R. G. Simpson, and M. C. Webb, 1959. Annual report for 1959, cooperative extension work in entomology, Lincoln, Nebraska.

Russell, R. M., and J. L. Robertson. **1979**. Programming Probit Analysis. Bull. Entomol. Soc. Am. 25: 191–193.

Sapkota, A. 2020. Serial dilution- definition, formula, calculator, procedure, uses. Microbe Notes. (https://microbenotes.com/serial-dilution/).

Scharf, M. E., B. D. Siegfried, L. J. Meinke, R. J. Wright, and L. D. Chandler. **2000**. Cytochrome P450-Mediated N-Demethylation Activity and Induction in Insecticide-Resistant and Susceptible Western Corn Rootworm Populations (Coleoptera: Chrysomelidae). Pestic. Biochem. Physiol. 67: 137–143.

Scharf, M. E., L. J. Meinke, B. D. Siegfried, R. J. Wright, and L. D. Chandler. **1999**. Carbaryl susceptibility, diagnostic concentration determination, and synergism for U.S. populations of western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 92: 33–39.

Siegfried, B. D., and C. A. Mullin. **1989**. Influence of alternative host plant feeding on aldrin susceptibility and detoxification enzymes in western and northern corn rootworms. Pestic. Biochem. Physiol. 35: 155–164.

Souza, D., A. V. Jiménez, G. Sarath, L. J. Meinke, N. J. Miller, and B. D. Siegfried. **2020a**. Enhanced metabolism and selection of pyrethroid-resistant western corn rootworms (Diabrotica virgifera virgifera LeConte). Pestic. Biochem. Physiol. 164: 165–172.

Souza, D., B. C. Vieira, B. K. Fritz, W.C. Hoffmann, J. A. Peterson, G. R. Kruger, L. J. Meinke. 2019a. Western corn rootworm pyrethroid resistance confirmed by aerial application simulations of commercial insecticides. Sci. Rpts. 9:6713. doi.org/10.1038/s41598-019-43202-w.

Souza, D., B. D. Siegfried, L. J. Meinke, and N. J. Miller. **2020b**. Molecular characterization of western corn rootworm pyrethroid resistance. Pest Manag. Sci. 77: 860–868.

Tabashnik, B. E. 2008. Delaying insect resistance to transgenic crops. Proc. Natl. Acad. Sci. USA. 105:19029–19030.

Tinsley, N. A., R. E. Estes, and M. E. Gray. **2014a**. Control of corn rootworm beetles with foliar-applied insecticides, 2013. Arthropod Manag. Tests. 39. doi.org/10.4182/amt.2014.F42.

Tinsley, N. A., R. E. Estes, and M. E. Gray. **2014b**. Control of corn rootworm beetles with foliar-applied insecticides and insecticide/fungicide combinations, 2013. Arthropod Manag. Tests. 39. doi.org/10.4182/amt.2014.F41.

Wangila, D. S., A. J. Gassmann, J. L. Petzold-Maxwell, B. W. French, and L. J. Meinke. 2015. Susceptibility of Nebraska western corn rootworm (Coleoptera: Chrysomelidae) populations to Bt corn events. J. Econ. Entomol. 108: 742-751.

Wechsler, S., and D. Smith. **2018**. Has resistance taken root in U.S. corn fields? demand for insect control. Am. J. Agric. Econ. 100: 1136–1150.

Wright, R. J., M. E. Scharf, L. J. Meinke, X. Zhou, B. D. Siegfried, and L. D. Chandler. **2000**. Larval susceptibility of an insecticide-resistant western corn rootworm (Coleoptera: Chrysomelidae) population to soil insecticides: laboratory bioassays, assays of detoxification enzymes, and field performance. J. Econ. Entomol. 93: 7–13.

Zhu, K. Y., G. E. Wilde, P. E. Sloderbeck, L. L. Buschman, R. A. Higgins, R. J. Whitworth, R. A. Bowling, S. R. Starkey, and F. He. **2005**. Comparative susceptibility of western corn rootworm (Coleoptera: Chrysomelidae) adults to selected insecticides in Kansas. J. Econ. Entomol. 98: 2181–2187.

Zhou, X., M. E. Scharf, G. Sarath, L. J. Meinke, L. D. Chandler, and B. D. Siegfried. **2004**. Partial purification and characterization of a methyl-parathion resistance-associated general esterase in Diabrotica virgifera virgifera (Coleoptera: Chrysomelidae). Pestic. Biochem. Physiol. 78: 114–125.

Zukoff, S. N., K. R. Ostlie, B. Potter, L. N. Meihls, A. L. Zukoff, L. French, M. R. Ellersieck, B. W. French, and B. E. Hibbard. 2016. Multiple assays indicate varying levels of cross resistance in Cry3Bb1-selected field populations of the western corn rootworm to mCry3A, eCry3.1Ab, and Cry34/35Ab1. J. Econ. Entomol. 109: 1387-1398.

Table 3.1. Background of western corn rootworm populations: location, date collected, and insecticide active ingredient exposure.

WCR Population	County	Year Collected	Insecticide
Control[1]	N/A	2020, 2021	Chlorpyrifos, bifenthrin, dimethoate
Bifenthrin[2]	N/A	N/A	Bifenthrin
FD1	Cuming	2019[3]	Chlorpyrifos, bifenthrin, dimethoate
FD2	Colfax	2020	Chlorpyrifos
FD3	Pierce	2020	Chlorpyrifos, bifenthrin
FD5	Cuming	2020	Chlorpyrifos, bifenthrin
FD6-Pre[3]	Boone	2019[5], 2020[6]	Chlorpyrifos, bifenthrin, dimethoate
FD6-Post[4]	Boone	2019[5], 2020[7]	Chlorpyrifos, bifenthrin
FD8	Cuming	2020	Chlorpyrifos, bifenthrin
FD10	Boone	2020	Chlorpyrifos, bifenthrin
FD12	Stanton	2019[5], 2020[7]	Chlorpyrifos, bifenthrin
FD16	Saunders	2020	Bifenthrin
FD17	Colfax	2020	Chlorpyrifos

[1]Crop Characteristic population.
[2]Laboratory population selected with bifenthrin (bif-R).
[3]WCR field population before insecticide application.
[4]WCR field population after insecticide application.
[5]All Insecticide bioassayed in one year.
[6]Chlorpyrifos bioassayed 2019
[7]Bifenthrin and dimethoate bioassayed 2020.
[8]Bifenthrin bioassayed 2020

Table 3.2. Western corn rootworm adult susceptibility of northeast Nebraska field populations to insecticide active ingredients, bifenthrin, estimated in 2020 and 2021.

Insecticide	Population	N[a]	Slope±SE	LC_{50} (95% CI)[b]	x^2(df)	RR_{50} (95% CI)[c]
Bifenthrin	Control[1]	210	2.08±0.32	0.40 (0.26-0.59)	5.71 (5)	1
	Bifenthrin[2]	240	2.58±0.29	0.93 (0.75-1.152)	4.55 (5)	2.31 (1.24-1.41)
	FD3	270	4.40±1.07	0.75 (0.54-0.91)	5.10 (6)	1.87 (0.90-1.12)
	FD5	270	5.60±1.52	0.42 (0.24-0.54)	6.66 (6)	1.06 (0.40-0.66)
	FD6-Pre[3]	300	1.42±0.26	2.35 (0.91-4.23)	9.011 (7)	5.88 (1.52-5.21)
	FD6-Post[4]	300	1.41±0.35	2.73 (0.60-4.86)	8.76 (7)	6.82 (0.99-5.98)
	FD8	270	2.11±0.26	0.41 (0.30-0.54)	2.94 (6)	1.03 (0.50-0.67)
	FD10	270	1.72±0.34	1.22 (0.33-2.21)	9.85 (6)	3.04 (0.55-2.72)
	FD12	180	2.75±0.43	0.19 (0.096-0.37)	16.22 (6)	0.48 (0.16-0.46)
	FD16	270	3.21±0.62	0.34 (0.17-0.48)	8.56 (6)	0.85 (0.28-0.60)

[a]Number of insects.
[b]µg/vial.
[c]Resistance ratio relative to control population. RR_{50} = (LC_{50} Population/ LC_{50} Control).
[1]Crop Characteristic population.
[2]Laboratory population selected with bifenthrin (bif-R).
[3]WCR field population before insecticide application.
[4]WCR field population after insecticide application.

Table 3.3. Western corn rootworm adult susceptibility of northeast Nebraska field populations to organophosphate active ingredients, chlorpyrifos and dimethoate, estimated in 2020 and 2021.

Insecticide	Population	N[a]	Slope±SE	LC_{50} (95% CI)[b]	x^2(df)	RR_{50} (95% CI)[c]
Chlorpyrifos	Control[1]	240	10.06±2.02	0.71 (0.60-0.81)	2.30 (5)	1
	FD1	238	1.61±0.17	1.19 (0.58-2.98)	22.92 (6)	1.67 (0.96-3.67)
	FD2	270	8.06±1.84	2.40 (1.80-3.05)	8.46 (6)	3.36 (2.99-3.76)
	FD3	270	2.70±0.60	1.71 (1.22-2.26)	5.10 (6)	2.40 (2.03-2.79)
	FD5	270	4.45±0.73	1.67 (1.37-1.96)	3.09 (6)	2.34 (2.28-2.41)
	FD6-Pre[2]	300	4.16±0.72	1.98 (1.57-2.41)	4.26 (6)	2.78 (2.61-2.97)
	FD6-Post[3]	300	3.44±0.98	3.72 (2.43-4.74)	5.19 (6)	5.22 (4.03-5.84)
	FD8	180	4.74±0.96	1.19 (0.87-1.57)	6.141(6)	1.68 (1.45-1.94)
	FD10	270	3.49±0.62	1.58 (1.20-1.96)	2.89 (6)	2.22 (2.00-2.41)
	FD12	270	4.74±0.86	1.16 (0.94-1.38)	2.53 (6)	1.62 (1.56-1.70)
	FD17	270	5.34±0.97	2.57 (2.15-3.02)	1.27 (6)	3.60 (3.57-3.71)
Dimethoate	Control	210	4.72±0.75	1.25 (1.05-1.47)	2.84 (4)	1
	FD1	240	2.20±0.24	1.84 (1.06-3.25)	13.33 (5)	1.47 (1.80-4.01)
	FD6-Pre	270	2.91±0.40	1.80 (1.40-2.21)	1.36 (6)	1.44 (2.33-2.73)

[a]Number of insects.

[b]μg/vial.

[c]Resistance ratio relative to control population. RR_{50} = (LC_{50} Population/ LC_{50} Control).

[1]Crop Characteristic population.

[2]WCR field population before insecticide application.

[3]WCR field population after insecticide application.

Table 3.4. Background of western corn rootworm populations: location, date collected, and formulated insecticide history per population.

WCR Population	County	Year Collected	Formulated Insecticide History			
			2017	2018	2019	2020
Control[1]	N/A	2020, 2021	N/A	N/A	N/A	N/A
Bifenthrin[2]	Perkins	2014	N/A	N/A	N/A	N/A
FD1	Cuming	2019	N/A	N/A	Bif	Soybean
FD2	Colfax	2020	Bif[3], Chlor[4]	Bif, Chlor	N/A	Bif, Chlor
FD3	Pierce	2020	Bif	N/A	Cobalt®[5]	Cobalt®
FD5	Cuming	2020	N/A	N/A	Bif, Chlor	Soybean
FD6-Pre	Boone	2019, 2020	Bif	Bif	Cobalt®	Chlor
FD6-Post	Boone	2019, 2020	Bif	Bif	Cobalt®	Chlor
FD8	Cuming	2020	N/A	N/A	N/A	N/A
FD10	Boone	2019	Bif	N/A	Cobalt®	Chlor
FD12	Stanton	2019	N/A	N/A	N/A	N/A
FD16	Saunders	2020	N/A	N/A	N/A	N/A
FD17	Colfax	2020	Unknown[6]	Soybean	N/A	Bif, Chlor

[1]Crop Characteristic population.
[2]Laboratory population selected with bifenthrin (bif-R).
[3]Bifenthrin
[4]Chlorpyrifos
[5]Chlorpyrifos+Lambda-cyhalothrin
[6]Unknown insecticide application history

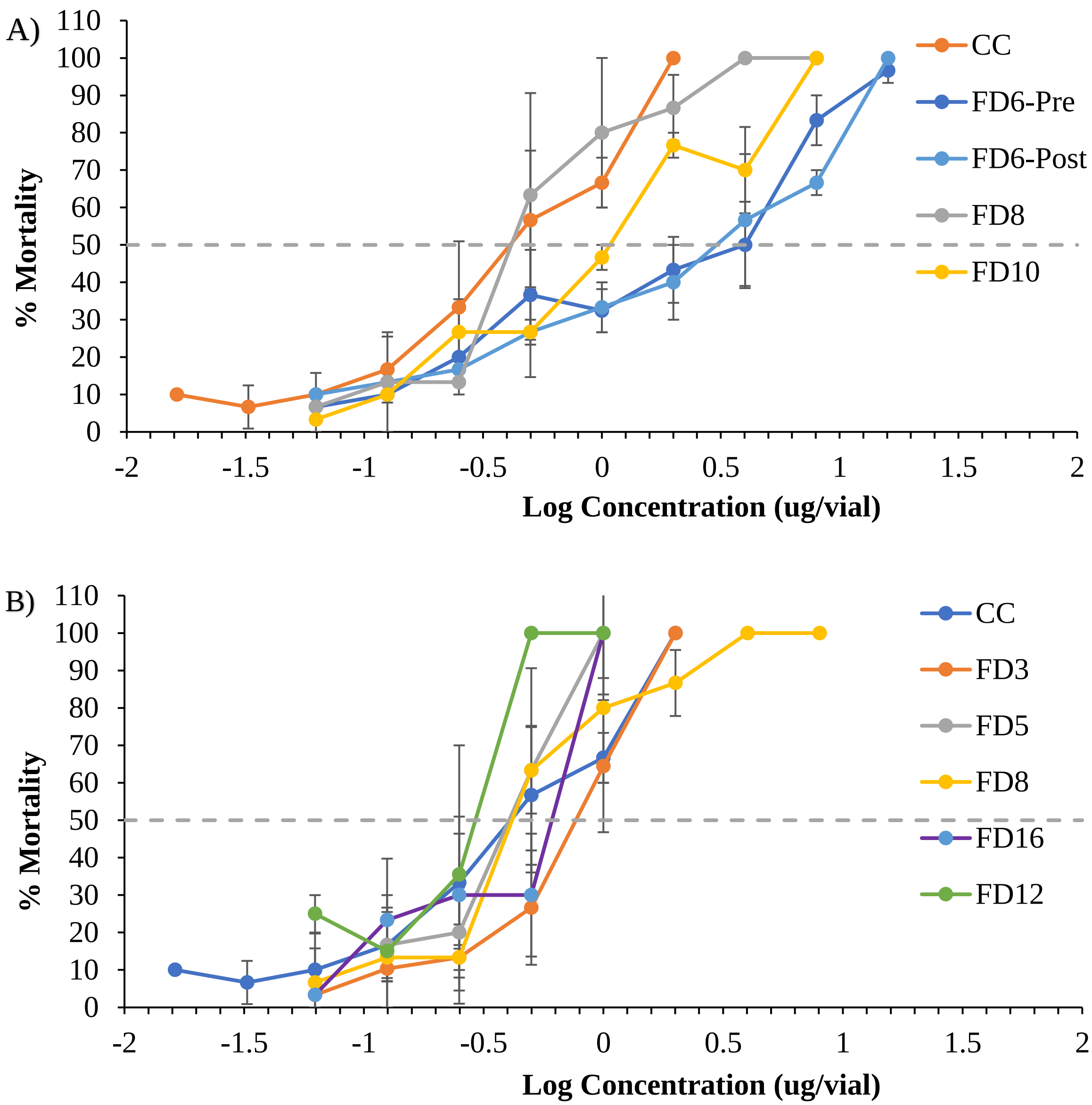


Figure 3.1. Bifenthrin dose-concentration curves of WCR field populations. A) Less susceptible WCR populations. Field F8 is a susceptible populations included for comparison. B) Susceptible WCR populations. Control populations same for both charts. The WCR % mortality at varying levels of log concentrations of bifenthrin. The grey threaded line across the chart represents 50% mortality.

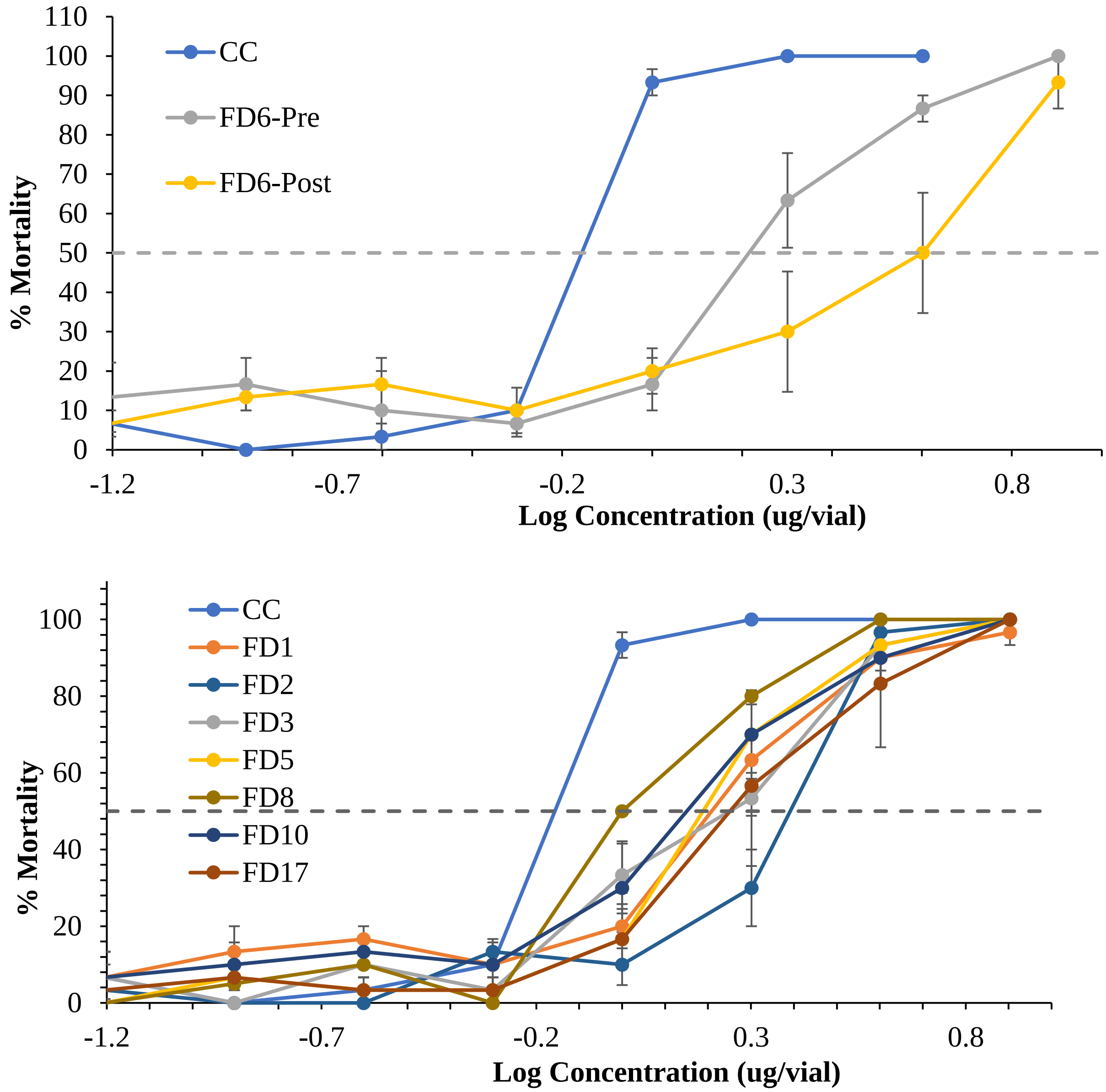


Figure 3.2. Chlorpyrifos dose-concentration curves of WCR field populations. A) Less susceptible WCR population B) Susceptible WCR populations. Control populations same for both charts. The WCR % mortality at varying levels of log concentrations of chlorpyrifos. The grey threaded line across the chart represents 50% mortality.

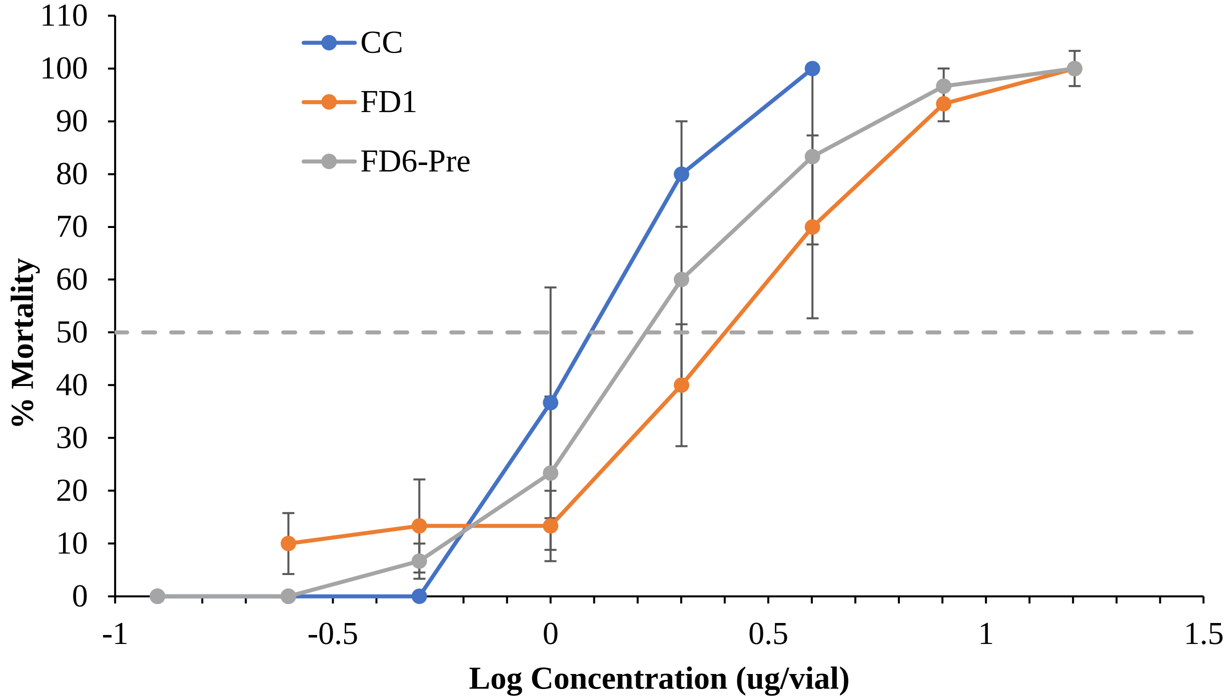


Figure 3.3. Dimethoate dose-concentration curves of WCR field populations. Only two field populations were bioassayed. The grey threaded line across the chart represents 50% mortality. Both field populations were susceptible to dimethoate.

APPENDIX I: WESTERN CORN ROOTWORM MEAN TOTAL SEX RATIO, 2019 AND 2020 FIELDS

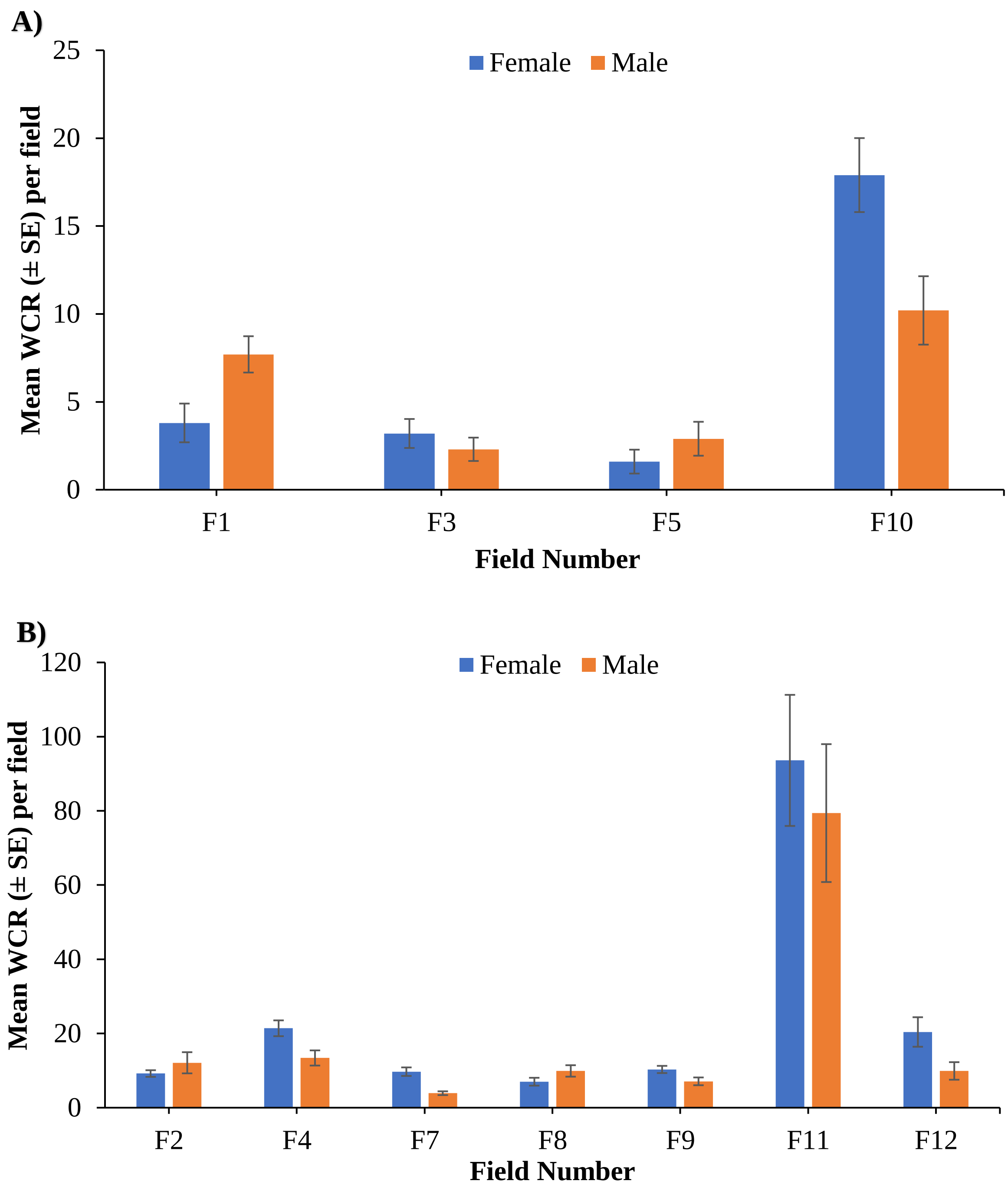
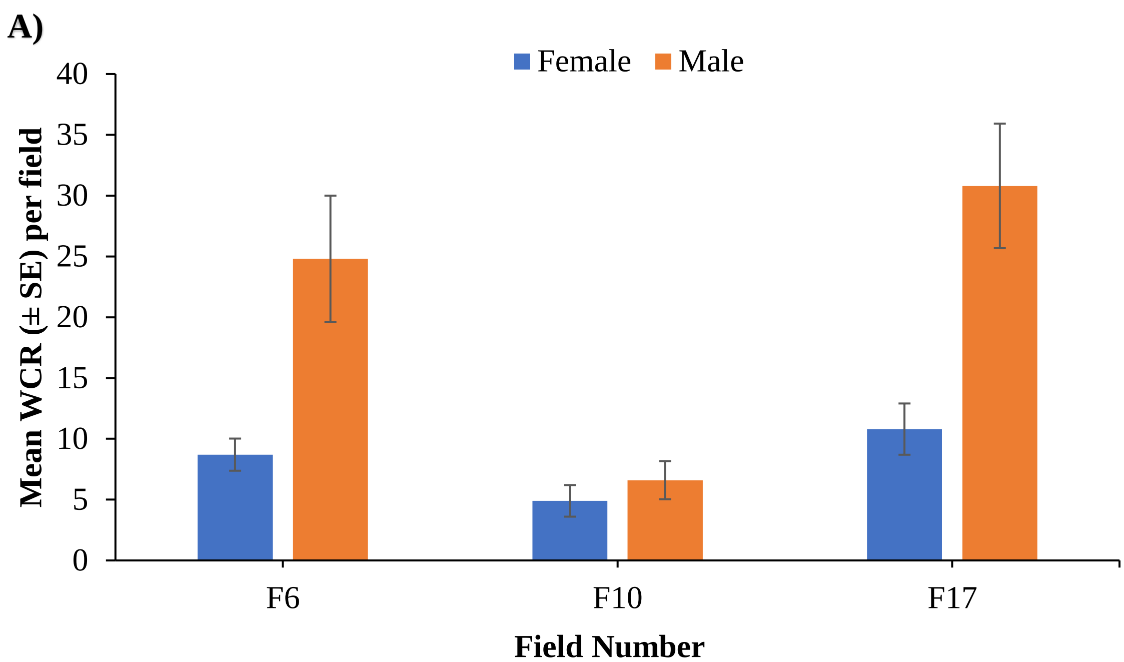


Figure 1. Sex ratio mean total western corn rootworm prior insecticide application sampled in 2019 fields. A) Before application foliar insecticide treated fields, B) Time period 01 untreated uncontrol fields. Individual bars represent mean ± standard error.



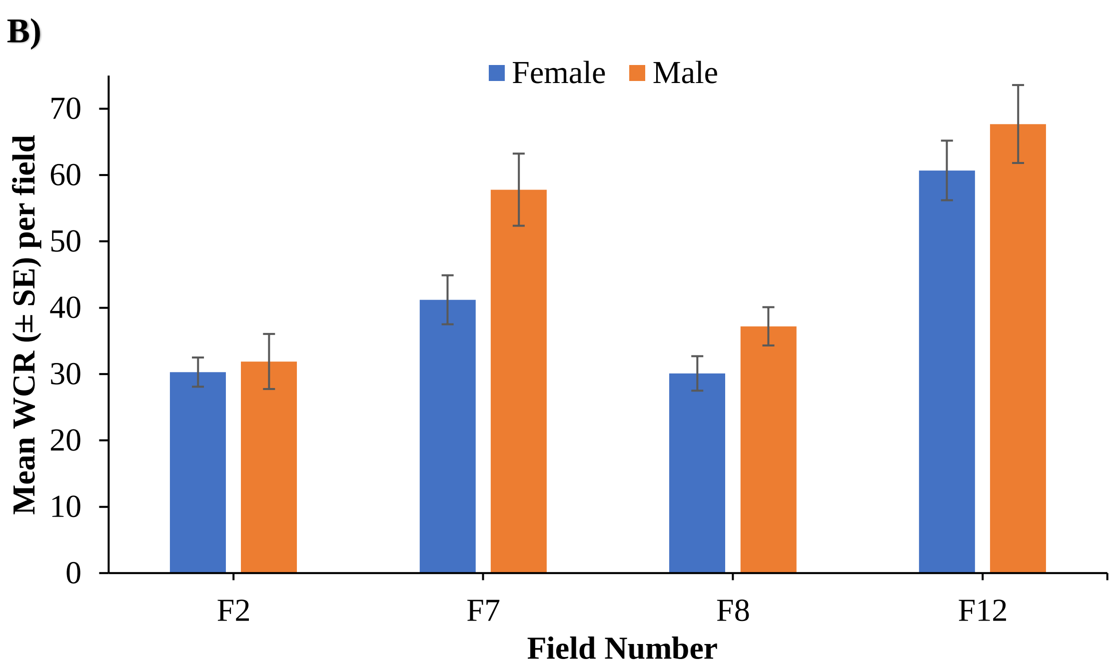


Figure 2. Sex ratio mean total western corn rootworm prior insecticide application sampled in 2020 fields. A) Before application foliar insecticide treated fields, B) Time period 01 untreated control fields. Individual bars represent mean ± standard error.

APPENDIX II: DOSAGE-CONCENRATION RANGES OF ACTIVE INGREDIENT INSECTICIDES FOR WCR POPULATIONS

Baseline Susceptibility of WCR Adults: The baseline susceptibility of WCR adults was determined by exposing 10 beetles of equal gender to 6-10 increasing concentrations depending on the insecticide to obtain a dose-response curve. Field populations, FD3, FD5, FD8, FD10, FD12, and FD16 were exposed to the same bifenthrin conc. range (0.0; 0.0625; 0.125; 0.25; 0.5; 1.0; 2.0; 4.0; 8.0 μg/vial); fields FD1 (0.0; 0.125; 0.25; 0.5; 1.0; 2.0; 4.0 μg/vial), FD6-Pre and FD6-Post (0.0; 0.0625; 0.125; 0.25; 0.5; 1.0; 2.0; 4.0; 8.0; 16.0 μg/vial), bif-R population (0.0; 0.125; 0.25; 0.5; 1.0; 2.0; 4.0; 8.0 μg/vial) concentration ranges were different. Chlorpyrifos conc. range (0.0; 0.0625; 0.125; 0.25; 0.5; 1.0; 2.0; 4.0; 8.0 μg/vial) was the same for all field populations. Dimethoate was only tested on 2 populations, FD1 (0.0: 0.25; 0.5; 1.0; 2.0; 4.0; 8.0; 16.0 μg/vial) and FD6-Pre (0.0; 0.125;0.25; 0.5; 1.0; 2.0; 4.0; 8.0; 16.0 μg/vial), due to lack of specimen available. The control population (C.C.) was exposed to different concentrations depending on the insecticide: bifenthrin (0.0; 0.01625; 0.0325; 0.0625; 0.125; 0.25; 0.5; 1.0; 2.0 μg/vial), chlorpyrifos (0.0; 0.0625; 0.125; 0.25; 0.5; 1.0; 2.0; 4.0 μg/vial), and dimethoate (0.0; 0.125; 0.25; 0.5; 1.0; 2.0; 4.0 μg/vial).